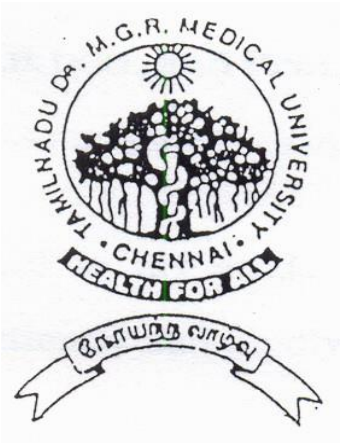
# A STUDY OF SOLUBLE FMS LIKE TYROSINE KINASE 1 AS PREDICTIVE MARKER OF PREECLAMPSIA IN PRIMIGRAVIDA

**Dissertation Submitted for**

**M.D DEGREE BRANCH - XIII**

**[BIO CHEMISTRY]**




**DEPARTMENT OF BIOCHEMISTRY**

**THANJAVUR MEDICAL COLLEGE,**

**THANJAVUR**

**THE TAMILNADU DR.MGR MEDICAL UNIVERSITY,**

**CHENNAI**

**APRIL - 2016**

# CERTIFICATE

This is to certify that dissertation titled "**A STUDY OF SOLUBLE FMS LIKE TYROSINE KINASE 1 AS PREDICTIVE MARKER OF PREECLAMPSIA IN PRIMIGRAVIDA"**is a bonafide work done by **Dr.K.MANJUKARTHIKEYANI** under my guidance and supervision in the Department of Biochemistry, Thanjavur Medical College, Thanjavur during her post graduate course from 2013 to 2016.

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**Remark of the Guide:**

The work done by **DR. K.MANJUKARTHIKEYANI** on **"A STUDY OF SOLUBLE FMS LIKE TYROSINE KINASE 1 AS PREDICTIVE MARKER OF PREECLAMPSIA IN PRIMIGRAVIDA"** is under my supervision and I assure that this candidate will abide by the rules of the Ethical Committee.

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# DECLARATION

I, **Dr.K.MANJUKARTHIKEYANI** hereby solemnly declare that the dissertation title "**A STUDY OF SOLUBLE FMS LIKE TYROSINE KINASE 1 AS PREDICTIVE MARKER OF PREECLAMPSIA IN PRIMIGRAVIDA"** was done by me at Thanjavur Medical College and Hospital, Thanjavur under the Supervision and Guidance of my Professor and Head of the Department **Dr.N.Sasivathanam, M.D(Bio).,DGO,**, This dissertation is submitted to the Tamil Nadu Dr. M.G.R Medical University, towards partial fulfillment of requirement for the award of M.D. Degree (Branch –XIII) in Biochemistry.

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## INSTITUTIONAL ETHICAL COMMITTEE

## CERTIFICATE

Approval No. : 006

This is to certify that The Research Proposal / Project titled

STUDY OF SOLUBLE FMS LIKE TYROSINE KINASE-I AS PREDICTIVE MARKER OF PRE-ECLAMPSIA IN PRIMIGRAVIDA

submitted by Dr. K. MANJU KARTHIKEYANI of

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was approved by the Ethical Committee.

Thanjavur

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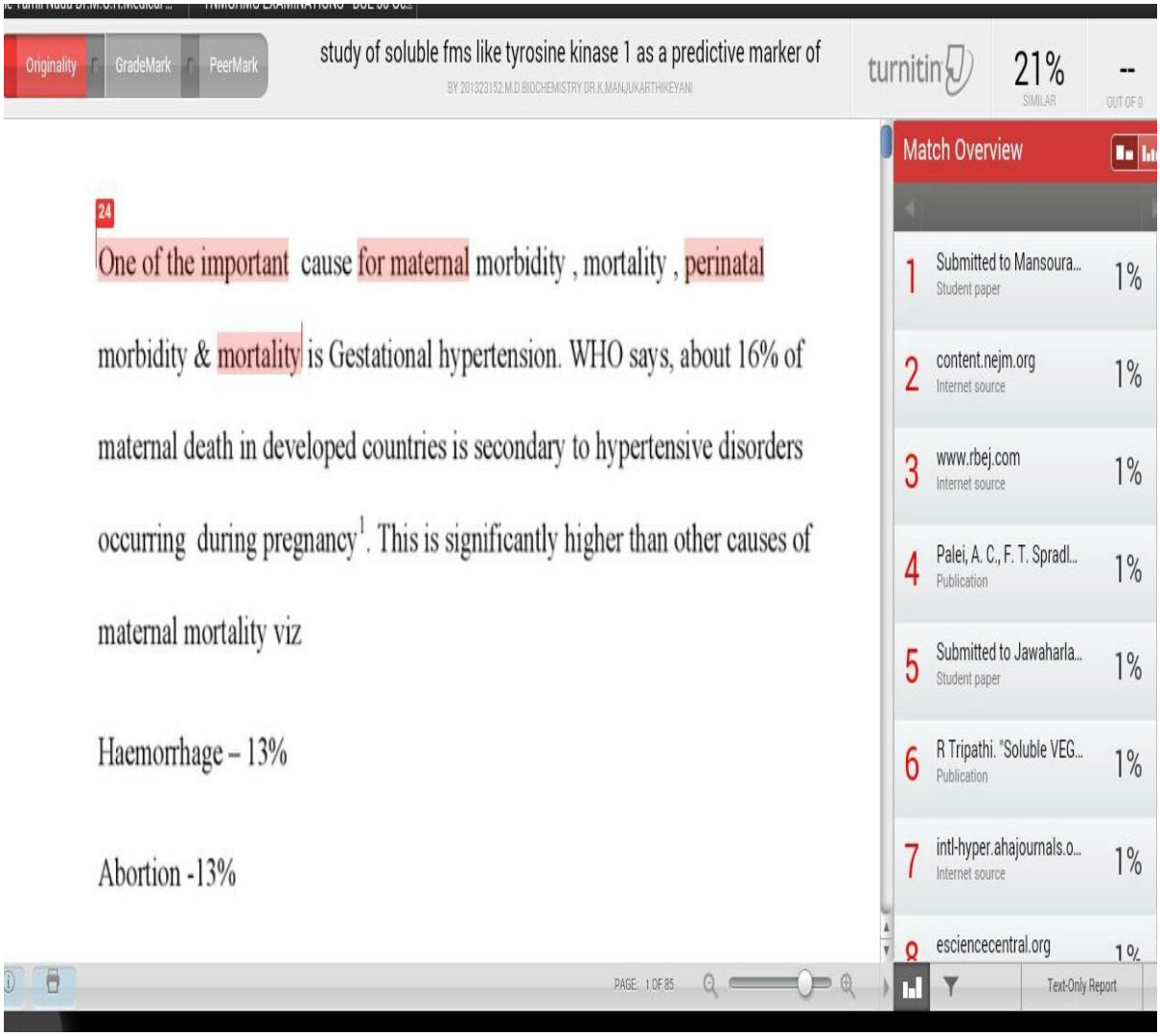

## ACKNOWLEDGEMENT

I am thankful to the Almighty who is the source of every good and perfect gift.

I am extremely grateful to **Dr.M.SINGARAVELU,** The Dean, Thanjavur Medical College for permitting me to do this dissertation at Thanjavur Medical College Hospital, Thanjavur.

I am indebted greatly to my Professor and Head of the Department, Department of Biochemistry, **Dr.N.SASIVATHANAM, M.D(Bio).,DGO,** who had inspired, encouraged and guided me in every step of this study.

I express my sincere gratitude to **Dr.KALARANI, M.D., DGO., HOD,** Department of Obstetrics & Gynecology for her valuable help.

I express my heartiest thanks to Dr.S.Ganesan,M.D(Bio),Associate Professor of Biochemistry and Dr.Josephine Latha, M.D(Bio).,Associate Professor, Department of Biochemistry, Thanjavur Medical College for their help and suggestions for performing my study.

I Sincerely thank my Assistant Professors Dr.R.Rajeswari, M.D(Bio).,DD., Dr.M.Ramadevi, M.D(Bio).,D.C.H., and Dr. P.Sunithapriya M.D(Bio)., Department of Biochemistry for their support during my study.

I owe my thanks to my co-post graduates for their support during the study.

I would like to acknowledge the assistance rendered by Non Medical assistants and the Technical staffs who helped me to perform the study.

I am grateful to all my patients and volunteers who participated in this study. I owe my special thanks to my family members for their moral support in conducting the study.

# CONTENTS

# ABBREVIATIONS

**WHO –** World Health Organisation.

**NHBPEP** - National High Blood Pressure Education Programs

**HT-** Hypertension

**LDH** – Lactate Dehydrogenase

**AST(SGOT)**– Aspartate Transaminase

**ALT(SGPT) --** Alanine Transaminase

**VEGF** -Vascular Endothelial Growth Factor

**PlGF-** Placental Growth factor

**sFlt-1-**Soluble fms-like tyrosine kinase

**KIR**- Killer-cell immunoglobulin-like receptor

**HLA –**Human Leucocyte Antigen

**PAPP-A** - Pregnancy associated Plasma Protein-A

**sVEGF-R1-** SolubleVascular Endothelial Growth Factor Receptor 1

**ELISA**- Enzyme-Linked Immunosorbent Assays

# STUDY OF SOLUBLE FMS LIKE TYROSINE KINASE 1 AS PREDICTIVE MARKER OF PRE ECALMPSIA IN PRIMIGRAVIDA


## ABSTRACT :

## INTRODUCTION :

Preeclampsia is pregnancy related disorder characterized by hypertension and proteinuria noticeable after 20 weeks of gestation. It is a leading cause of maternal and foetal mortality and morbidity. Preeclampsia affects 3-5% of all the pregnancies . Soluble fms like tyrosine kinase 1, an anti angiogenic factor is involved in vasoconstriction and endothelial dysfunction responsible for the development of preeclampsia.

## AIM & OBJECTIVES :

### PRIMARY OBJECTIVE

To find the association between soluble fms like tyrosine kinase 1 and incidence of preeclampsia in primi gravida.

### SECONDARY OBJECTIVE

1.To determine the predictive value of soluble fms like tyrosine kinase 1 for preeclampsia.

2.To determine whether early screening can be done for preeclampsia using soluble fms like tyrosine kinase 1

## MATERIALS AND METHODS :

This is a prospective study conducted in Thanjavur medical college hospital and allied institutions. Blood and urine samples were collected from primigravida patients attending department of Obstetrics and Gynecology, TMCH/RMH and analyzed for soluble fms like tyrosine kinase 1, liver enzymes , platelet count and urine for protein. Soluble fms like tyrosine kinase 1 is estimated by sandwich elisa method

### INCLUSION CRITERIA:

All Primi Gravida pateints attending department of obstetrics and gynecology, TMCH/RMH

### EXCLUSION CRITERIA

Known cases of Diabetic , Hypertensive and Hepatic disease

Known patients with proteinuria

Known patients with other organ dysfunction.

## DISCUSSION:

Increase in placental derived soluble fms-like tyrosine kinase 1 (sFlt-1) is responsible for the signs and symptoms of Preeclampsia, and increased levels of these circulating markers are associated with Preeclampsia. In our study, we focused only on primigravida, because nulliparity itself is a main risk factor for the development of Preeclampsia. If we look into distribution of study population according to Preeclampsia and SFLT levels through Pearson's Chi-square with continuity correction( 69.348, p value: <0.001), there is a statistically significant association between elevated SFLT levels and occurrence of Preeclampsia with 86.4% of Pre-eclampsia & Eclampsia patients showing elevated SFLT levels .

## CONCLUSION

From this study, It is found that the primigravida with increased serum sFlt levels developed Preeclampsia in the later pregnancy and few of them developed Eclampsia also . So , serum sFlt levels can be used to predict the occurrence of both Preeclampsia and also progression to Eclampsia.

**KEY WORDS :**

Preeclampsia, Eclampsia ,Primigravida, Soluble fms-like tyrosine kinase 1,

## INTRODUCTION

One of the important cause for maternal morbidity, mortality and perinatal morbidity & mortality is Gestational hypertension. WHO says, about 16% of maternal death in developed countries is secondary to hypertensive disorders occurring during pregnancy[1]. This is significantly higher than other causes of maternal mortality viz

Haemorrhage – 13%

Abortion -13%

Sepsis -2%

Gestational hypertension is a significant factor accounting for 10 to 38% maternal deaths in South Asia. In India, the maternal& perinatal mortality due to Preeclampsia has been reported to be 12% & 4.76% respectively[2,3]. Even in developed countries the case fatality rate has been reported to be 1.8% with Eclampsia and further 35% of women develop major complications.[4]

Gestational hypertension is a disorder of abnormal placentation developing around 12 weeks of pregnancy. The main consequence of gestational hypertension is placental ischemia which leads to endothelial dysfunction , responsible for development of clinical symptoms and complications.

"Gestational hypertension" is diagnosed when a pregnant women comes with a blood pressure of more than or equal to 140/90 mmHg for the first time in mid pregnancy [20 weeks] but proteinuria not identified.

In this, about half of them subsequently develop Preeclampsia syndrome characterised by proteinuria, thrombocytopenia and other symptoms and signs of end organs injury.

Early detection of gestational hypertension and giving adequate antenatal care will definitely reduce both maternal and perinatal morbidity &mortality.

An imbalance between angiogenic and anti angiogenic factors is responsible for etiopathogenesis of gestational hypertension. The biochemical factors for abnormal angiogenesis are "Placental growth factor" & "Soluble fms like tyrosine kinase 1". These factors are more specific than measuring blood pressure and proteinuria[6].

"Soluble vascular endothelial growth receptor 1" otherwise called "soluble fms like tyrosine kinase 1" antagonises vascular endothelial functions leading to hypertensive disorders of pregnancy[7].

In this study ,serum level of Soluble fms like tyrosine kinase 1 is evaluated in cases of primigravida with Preeclampsia

# AIM & OBJECTIVES :

**PRIMARY OBJECTIVE**

To find the association between soluble fms like tyrosine kinase 1 and incidence of preeclampsia in primi gravida.

**SECONDARY OBJECTIVE**

1.To determine the predictive value of soluble fms like tyrosine kinase 1 for preeclampsia.

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# REVIEW OF LITERATURE

# REVIEW OF LITERATURE

"Hypertensive disorders of pregnancy" are one of the common medical complication occurring in pregnancy, accounting for 20% of all maternal deaths in India. Gestational hypertension is one of the unique disorder in pregnancy which disappears with delivery. Gestational hypertension usually occurs after 20th week of gestation but the underlying mechanisms start as early as 8-18 weeks of pregnancy[30,31]. Gestational hypertension is one of the most common condition responsible for both maternal and perinatal morbidity and mortality[9].

The working group of NHBPEP [National high blood pressure education programs2000] classified hypertensive disorders complicating pregnancy into four types [5].

1. Gestational hyper tension,
2. Preeclampsia & eclampsia syndrome,
3. Preeclampsia syndrome superimposed on chronic hypertension.
4. Chronic hypertension

**Gestational hypertension**

Increased blood pressure occurring in the second trimester of pregnancy for the first time with the no proteinuria defines gestational hypertension.

**Gestational hypertension -Criteria**

The increased blood pressure should be present on at least 2 occasions, at least 4 hrs apart but within in a maximum of one week period .

1. Systolic pressure ≥140mmHg and diastolic ≤90mmHg for the 1st time during pregnancy.

2. Proteinuria not seen

3. Hypertension returns to normal before twelve weeks of postpartum.

4. Absent intrauterine growth retardation and oligohydramnios by ultrasound

**Pre-eclampsia and eclampsia**

Onset of new hypertension and proteinuria after 20th week of pregnancy defines Preeclampsia. It leads to abnormalities of the coagulation system, cerebral ischaemia, renal failure, and disturbed liver function[10].

Preeclampsia is a disease with multisystem involvement and it is characterized by proteinuria of ≥ 300 mg in 24 hrs urine sample. Clinically, Preeclampsia is classified as mild and severe. When signs and symptoms of end-organ injury are seen along with severe hypertension & proteinuria, it is severe Preeclampsia .

Also HELLP syndrome characterized by Low Platelets ,Elevated Liver enzymes and Hemolysis represents one form of severe Preeclampsia. If the above findings are absent, Preeclampsia is called as mild. When

Preeclampsia is associated with seizures it is called Eclampsia, which is seen in 0.1% of all pregnancies, while Preeclampsia is seen in 3–5% of pregnancies.

Both proteinuria and HT (hypertension) affects the endothelium which is main target of the disease. The hypertension of Preeclampsia is mainly characterized by decreased arterial compliance and peripheral vasoconstriction[24,25]. In patients having overt proteinuria around 10 percent develop Eclampsia.

**Criteria for Preeclampsia in pregnant women**

Minimum criteria

1. BP ≥140/90mmHg
2. Proteinuria ≥300mg /24 hrs or > 1+ with dipstick

**Criteria for increased certainty for Preeclampsia in pregnant women**

1.BP ≥ 160/110mmHg

2. Serum creatinine >1.2 mg/dl

3. Platelets <100000/mm3

4.Proteinuria ≥ 2g /24 hrs or >2+dipstick

5.Increased Lactate Dehydrogenase

6.Elevated liver enzymes levels [ALT&AST]

7.Maternal symptoms are persistent headache , epigastric pain and visual disturbances [5]

**HELLP SYNDROME -CRITERIA**

| | |
|---|---|
| **Hemolysis**– | Serum bilirubin ≥1.2mg per dl<br>LDH more than 600 U per L<br>Peripheral blood smear shows damaged<br>Erythrocytes like schistocytes and burr cells |
| **Liver enzymes** | ALT increased<br>AST increased |
| **Platelet count** | less than 100,000 per $mm^3$<br>Or<br>Class 1: ≤50,000 per mm3<br>Class2 : 50,000 but ≤ 100,000 per mm3<br>Class3 : > 100,000 but < 150,000 per mm3 |

**Pre-eclampsia superimposed on chronic hypertension**

Any new onset proteinuria after 20 weeks of pregnancy in women with chronic hypertension heralds the onset of Preeclampsia which is superimposed on chronic hypertension.

About 30% of women with chronic hypertension may develop Preeclampsia.In uncomplicated chronic hypertension, there is no proteinuria

**Criteria for superimposed Preeclampsia on hypertension in pregnant women**

1. Newer onset proteinuria more than or equal to 300 mg in a 24 hrs urine sample of hypertensive pregnant women after 20 weeks of pregnancy .

2. Any sudden increase in proteinuria in patients with pre-existing excretion of urine protein, sudden increase in blood pressure or decrease in platelet count less than 1,00,000/$mm^3$ also indicates superimposed Preeclampsia on hypertension .

**Chronic hypertension**

Any pregnant patient coming before 20 weeks of pregnancy with a Blood pressure **more than or equal to**140/90 mmHg define chronic hypertension in pregnant women. It is also associated with raised perinatal and Maternal morbities.

**Chronic hypertension in pregnant women-Criteria**

1.Blood Pressure more than or equal to140/90mmHg before the onset of pregnancy or before 20 wks pregnancy ( not attributed to gestational trophoblastic disease).

2. Hypertension persistent after 12 weeks delivery even when diagnosed after 20 weeks gestation.

Another rare entity is atypical Preeclampsia seen with all aspects of the syndrome but without hypertension or proteinuria, or both [sibai and stella,2009][38].

**Indicators of severity of Preeclampsia**

Headaches and visual disturbances such as scotomata can be premonitory symptoms of Eclampsia. Epigastric or right upper quadrant pain is due to hepatocellular necrosis or ischemia probably secondary to stretching of Glissons'capsule. This pain frequently associated with elevated transaminase levels.

Thrombocytopenia is also an important finding indicating worsening of Preeclampsia . It is due to platelet activation and aggregation as well as microangiopathic haemolysis induced by vasospasm. Eclampsia incidence has decreased over the years due to adequate prenatal care.

**INCIDENCE**

Hypertensive disorders of pregnancy occur in about 12-22% of pregnant women which is more prevalent (25% of nulliparous women are complicated by hypertensive disorders)[11].

Gestational hypertension is increased blood pressure occurring during pregnancy or within first 24 hours of delivery without features of Preeclampsia

or pre-existing hyper tension[8]. Incidence in nulliparous & multi parous women is between 6%, 29% and 2%, 4% respectively.

The incidence increases with advanced age of gestation, with more than 50% of cases occurring in term (around 37 weeks pregnancy). Maternal and perinatal morbidities are increased.

Early onset gestational hypertension is often complicated by severe Preeclampsia. Preeclampsia is considered primarily as a disease of primigravida[8]. Primigravida are also at higher risk of seizures. Seizures can occur either antepartum (38%) &intrapartum (18%) or postpartum (44%) Antepartum seizures are more dangerous than postpartum seizures[16].

Preeclampsia produces severe complications in the mother like acute renal failure, placental abruption, hepatic failure, intracranial haemorrhage, disseminated intravascular coagulation, cardiovascular collapse and in fetus prematurity, "Intrauterine fetal growth retardation" (IUGR), Intrauterine fetal death[12]. Many Studies have shown that maternal and fetal mortality are significantly higher in nulliparous women with hypertensive disorders[13].

**Epidemiology of gestational Hypertension:**

Preeclampsia is one of the significant public health threat in both maternal and perinatal morbidity and mortality globally.

It has been estimated that, 10 to 15% of direct maternal mortality (ie, due to obstetric complications of pregnancy) are secondary to Preeclampsia & Eclampsia[44] . In the United States of America, Preeclampsia and Eclampsia are one of leading cause of maternal death, along with thromboembolism, haemorrhage and cardiovascular problems[45-47]. There is about one maternal death per 100,000 live births due to Preeclampsia /eclampsia, with a case-fatality rate 6.4 deaths per 10,000 cases[48, 49]. In Netherlands, over a study period of 12 years. Preeclampsia was found to be the most common cause of maternal mortality, with 3.5 deaths per 100,000 live births[50]. It occurs in about 2.5% of pregnant Japanese women [51].

In India, these hypertensive disorders account for 31% of maternal deaths, with 24.7% secondary to Eclampsia[64].

**RISK FACTORS ;**

The risk factors include [i] Family history of Preeclampsia, [ii]Primigravida, [iii]Higher incidence in pregnant women with younger than 20and older than 40 years,[iv] Twin pregnancy, triplets or more [v]Prolonged interval between pregnancies [vi]Obesity [vii] Gestational diabetes have a great risk of developing Preeclampsia as the pregnancy progresses and [viii]History of medical conditions such as chronic hypertension, migraine , rheumatoid arthritis

,kidney disease , diabetes, periodontal disease during pregnancy and urinary tract infections [21].

Other factors include Auto immune conditions, Environmental, Socioeconomic and Seasonal influences [ Palmer, 1999][68].

Two karyotypic anomalies hydatiform mole and trisomy 13 are known to be associated with a risk for Preeclampsia. The association between trisomy 13 and Preeclampsia was first suggested in 1987 by Redman et al[4]. It was confirmed by a larger retrospective study published in 1992 by Tuohy and James[43].

Advanced maternal age and body mass index are associated with increased risk for late Preeclampsia . Advanced maternal age alone is an independent risk factor for Preeclampsia[28] . LCY Poon et al [20] study shows that the risk of late Preeclampsia and gestational hypertension increases by 4% for every year over the age of 32 years & by 10% for every 1 kg $m^2$ above 24kg $m^2$

Obesity is characterized by expanded blood volume, increased cardiac output and an increase in oxygen consumption. Stroke volume and cardiac out put are increased to meet the increased metabolic demands. So hypertension results when systemic resistance fails to decrease as cardiac output increases[19]. An elevated cardiac output in obese patients cannot tolerate further increase secondary to pregnancy associated haemodynamic alterations . So

they may develop hypertension with increased blood flow, exacerbating the endothelial injury leading to sequelae of Preeclampsia [19].

**Risk factors that can be assessed at first visit[14]**

| | |
|---|---|
| **History**<br>Age<br>Parity<br>Previous pre-eclampsia<br>Family history of pre-eclampsia<br>Multiple pregnancy<br>Pre-existing medical conditions:<br>Insulin dependent diabetes (IDDM)<br>Chronic hypertension<br>Renal disease | Autoimmune disease<br>Antiphospholipid syndrome<br>Time between pregnancies<br>Body mass index (BMI)<br><br>**Examination**<br>Blood pressure<br>Proteinuria |

South Asians have a high prevalence of insulin resistance, elevated triglycerides and low high density lipoprotein levels [20]. The metabolic abnormalities (glucose intolerance, hyperinsulinemia, hyper lipidemia) related with insulin resistance are also observed in women with gestational hypertension . The markers of insulin resistance include Plasminogen Activator Inhibitor-1, Leptin, and TNF-alpha[33].

Assisted reproductive techniques increase the risk for Preeclampsia .because the maternal serum concentration of pregnancy associated Plasma Protein –A is reduced. So there is extensive evidence of linking low serum levels of pregnancy associated Plasma Protein –A with subsequent development Preeclampsia[20].

## RISK FACTORS ASSESSMENT WITH VARIOUS STUDIES[14]

### Age

Most of the studies suggest that women aged ≥ 40years particularly those with pre-existing chronic disease has double the risk of developing preeclampsia, whether they were multiparous or primiparous. (RR 1.68, 95% confidence interval, 1.23 to 2.29, and 1.96, 1.34 to 2.87, respectively) [61].

### Parity

Nulliparae are three times at risk for preeclampsia . (RR 2.35, 95% confidence interval 1.80 to 3.06) [14] .

### Previous preeclampsia

History of preeclampsia in the first pregnancy is a risk factor for development of Preeclampsia /Eclampsia in second pregnancy and risk increases by **seven times in the 2nd pregnancy** (RR 7.19, 95% confidence interval 5.85 to 8.83) [14].

**Family history in pre-eclampsia**

History of preeclampsia in the family is a risk factor for development of Preeclampsia /eclampsia in women. The risk **increases by three times** (RR 2.90, 95% confidence interval 1.70 to 4.93)[14].

**Multiple pregnancy**

Women who have twin pregnancy will have **triple the risk of developing preeclampsia** (RR 2.83, 95% confidence interval 1.25 to 6.40) [33].

**Pre-existing medical conditions**

Type 1 Diabetes— In patients with type 1 DM, the risk of developing preeclampsia raises by 4 times if **diabetes is present before the onset of pregnancy** (RR 3.56, 95% confidence interval 2.54 to 4.99)

Pre-existing chronic Hypertension—one case-control study which is population based pointed out that, **there is increased prevalence of chronic hypertension** in women who developed Preeclampsia than those who did not (12.1% v 0.3%)[14,37].

Viral infections Parvo virus infections during pregnancy leads to over expression of an anti angiogenic factor "soluble fms like tyrosine kinase 1 " responsible for development of Preeclampsia[22] .

**Is There Any Protection For Preeclampsia**

Smoking during pregnancy causes a variety of adverse outcomes, but it has been associated with a reduced risk of developing hypertension during

pregnancy. It was speculated that potentially effective agents in tobacco smoke such as thiocyanate may have a hypotensive effect[62], and nicotine was found to inhibit production of fetal thromboxane A2 [63].

**Physiologic changes in normal Pregnancy:**

In pregnancy ,the cardiac output and blood volume increases by about 40% and 50% respectively.

**Serum chemistry changes associated with pregnancy:**

Blood Urea Nitrogen decreases to < 10 mg/dl

Creatinine decreases to < 0.7 mg/dl

Uric acid decreases to< 4.0 mg/dl

Serum albumin & haematocrit also decreases (due to hemodilution) during pregnancy.

Plasma osmolality and sodium are decreased during pregnancy.

Arterial blood gas analysis (ABG)show decreased $pCO_2$ secondary to progesterone induced hyperventilation[29].

**Hemodynamic and Volume Alterations in Preeclampsia.**

There is increase in cardiac after load and systemic vascular resistance in women with Preeclampsia. There is reduction in the levels of renin and aldosterone II in preeclamptic patients. As the preeclamptic patients have

reduced plasma volume it becomes essential to maintain high levels of renin and aldosterone II

**Hormonal changes:**

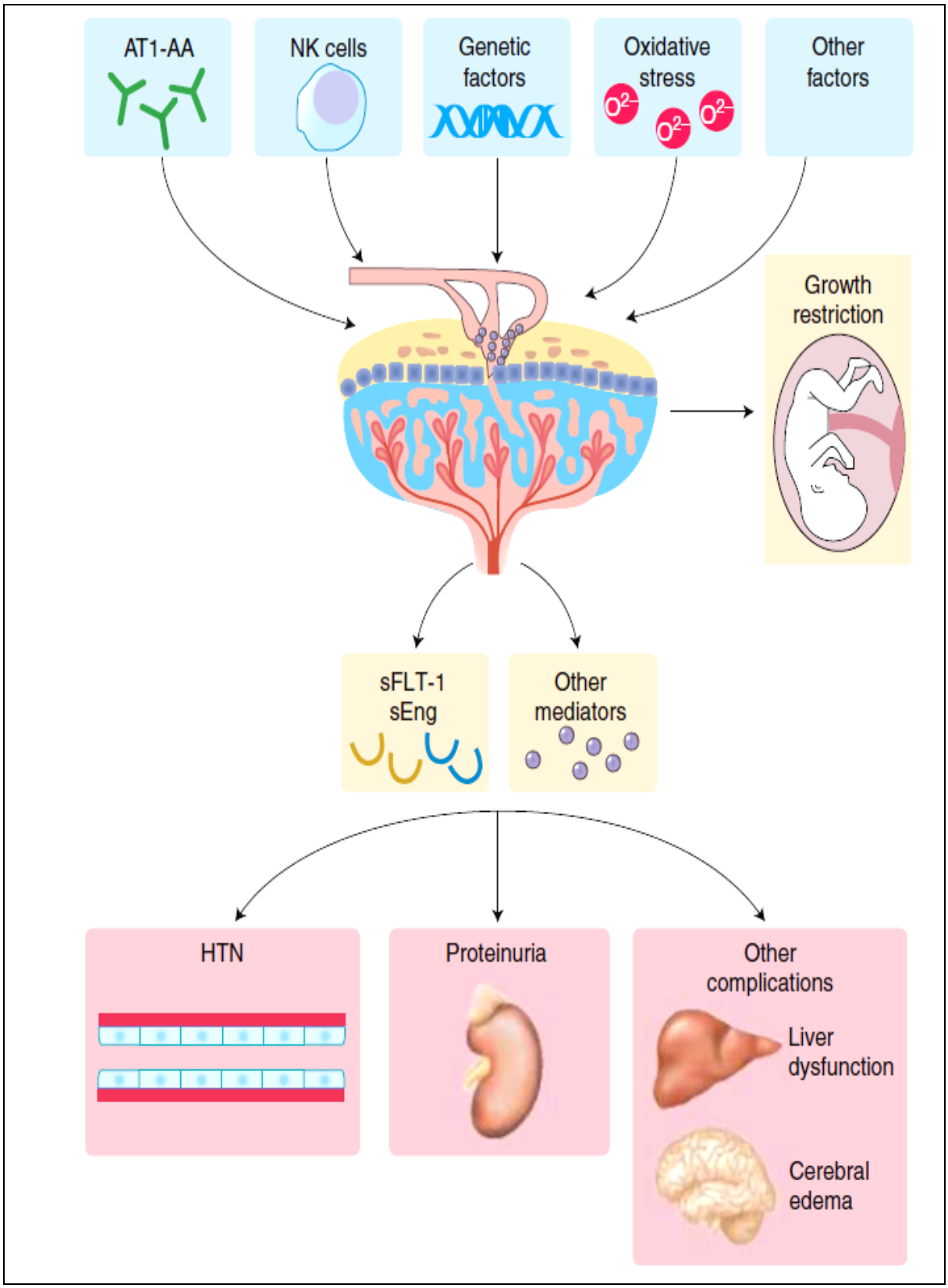
Blood volume is mainly regulated by circulating levels of catecholamine, aldosterone and renin angiotensin system. There is a paradoxical raise in all these hormones during pregnancy [32].

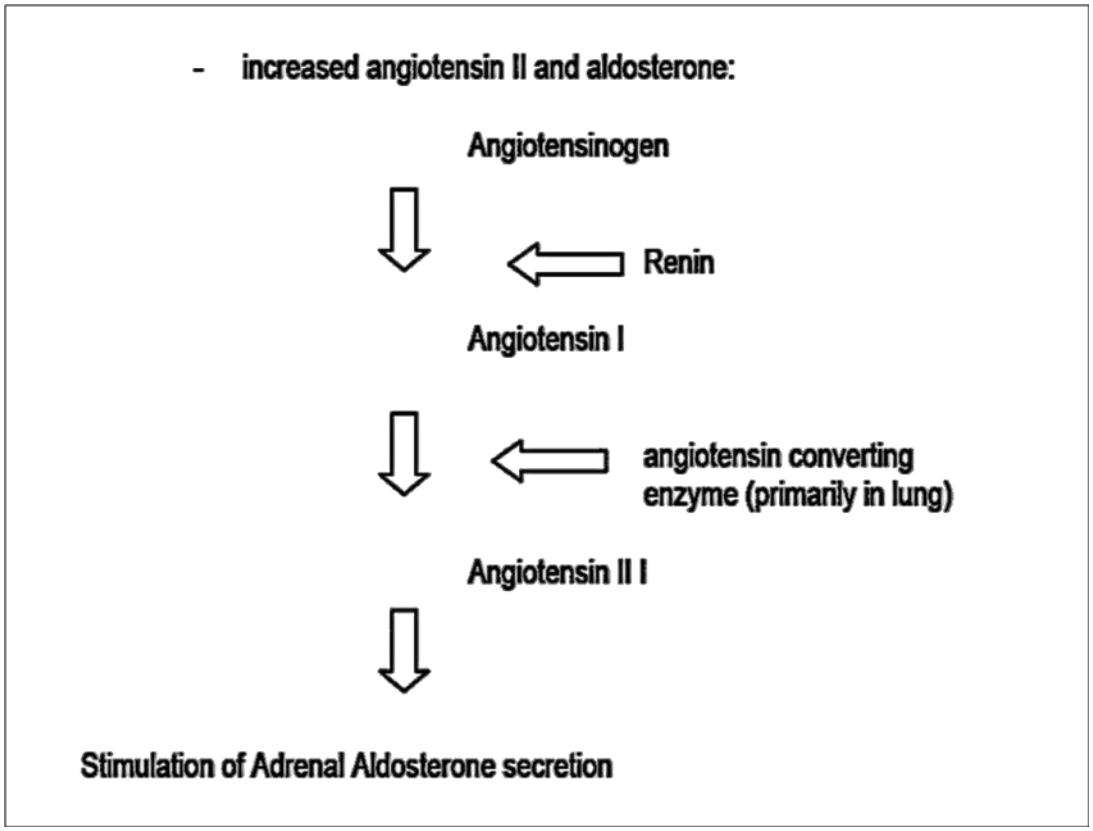
Increased renin secretion from utero placental origin stimulates the adrenal aldosterone secretion. This rise in all levels leads to a decreased sensitivity to this vasoconstrictor, related to the marked increase in vasodilatory Prostaglandins in pregnancy.

**Renal Alterations in Preeclampsia.**

The GFR and renal blood flow are decreased in preeclamptic women. If proteinuria develops, a kidney biopsy if undertaken will typically show glomerular endotheliosis and cortical necrosis. This lesion is characteristic in preeclamptic women. Injury to the endothelium play a pivotal role in the pathology of Preeclampsia.[32].

**PATHOGENISIS OF PREECLAMPSIA**





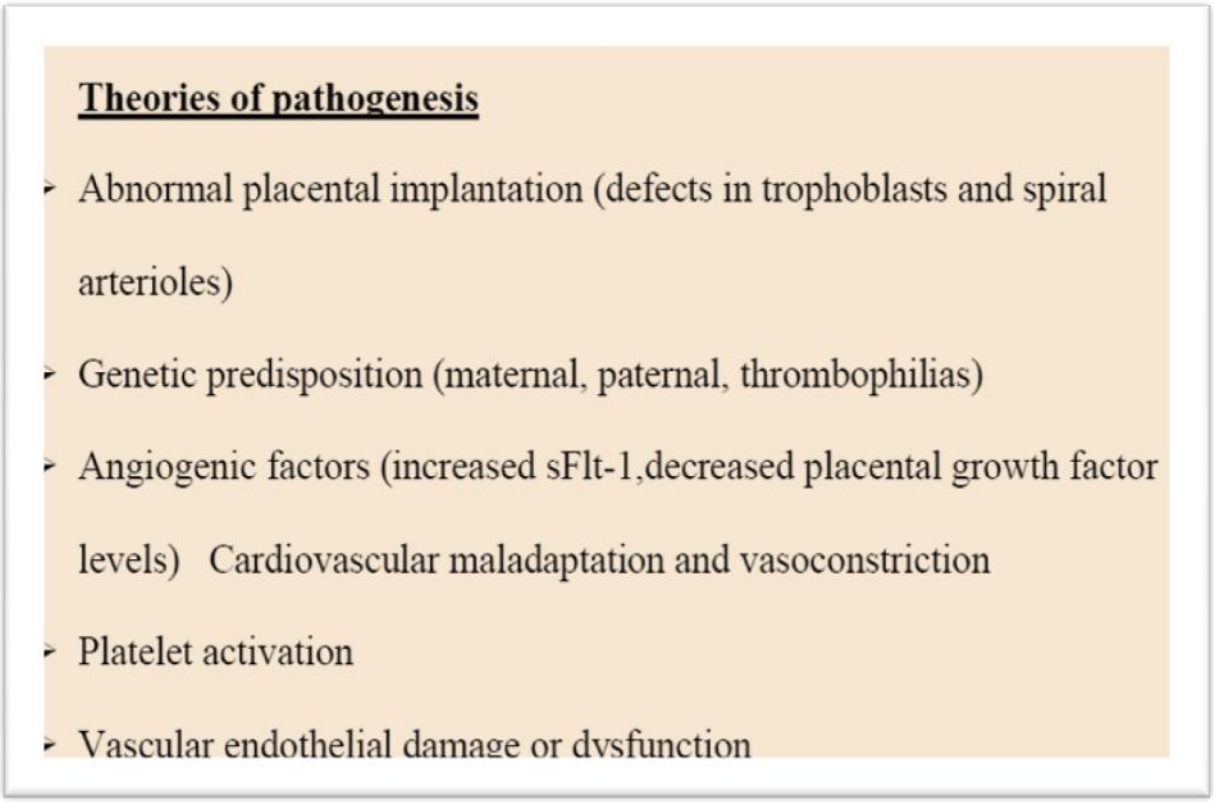

**Role of vasodilatory prostaglandins ($PGE_2$ and $PGI_2$):**

Vasodilatory prostaglandins [$PGE_2$ and $PGI_2$] secreted from placenta as well as other vascular endothelial origin and also decreased production of the vasoconstrictor prostaglandin thromboxane. Prostaglandins are derived from the precursor arachadonic acid and its structure consists of 20 carbon fatty acids with a cyclopentane ring. $PGI_2$ (prostacyclin) is a potent vasodilator and inhibitor of platelet aggregation. Thromboxane causes vasoconstriction and platelet aggregation.

**Placental Ischemia**

The important pathophysiology in Preeclampsia is altered utero placental blood flow. Most studies about the pathogenesis of this condition suggest that a reduction in uterine blood flow is the main confounding factor. There is uniformly abnormality in the placenta of preeclamptic patients. The primary pathology appears to be at the maternal fetal interface and is characterized by poor trophoblastic invasion of the uterus. The endovascular invasion of the spiral arteries is incomplete. Specifically, the failure of the cyto trophoblasts to penetrate deep and cause a "widening of the pathway" appears to explain the relative reduction in utero placental blood flow.[32]

**PATHOPHYSIOLOGY**



# NORMAL AND PRE ECLAMPSIA PLACENTAL STRUCTURE

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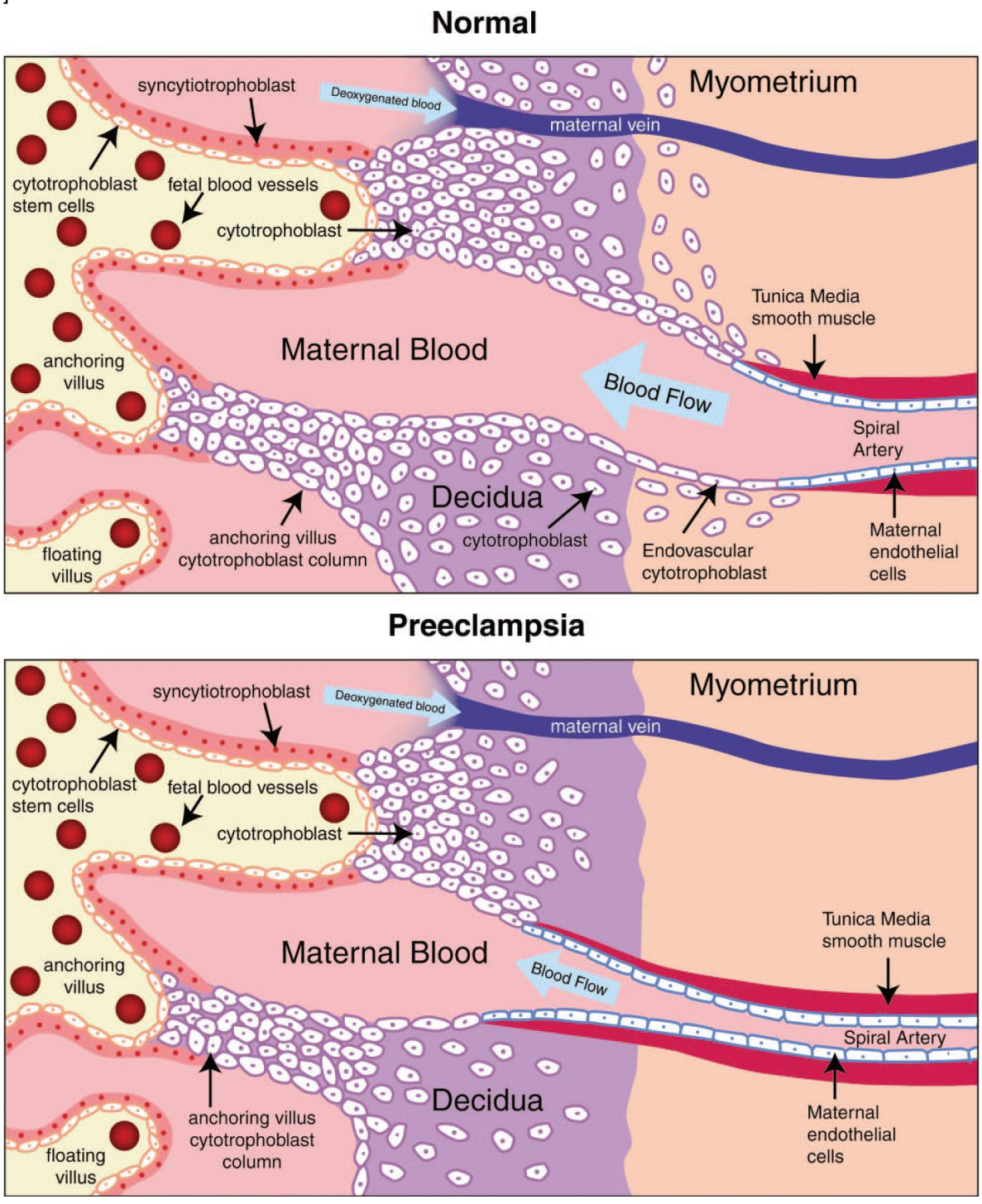


**Placental structure**

In human placenta is divided into

Basal part(maternal surface)

Chorionic part (fetal surface)

Amnion and the underlying chorion are membranes

Gas and nutrients exchange takes place actively in terminal villous unit. Amnion, chorion are the 2 membranes that cover chorionic plate. The chorionic arteries are collected at the umbilical cord [Kraus et al 2004][58].

**Formation of blood vessels**

The first step in the production of blood vessels is the formation of branched vascular plexus. The primary vascular branched plexus forms by several processes, including the initial assembly of vascular precursor cells called angiogenesis

Vascular plexus formation depends on the regulated expression of vascular endothelial growth factor (VEGF) by non-endothelial cells. This expression of VEGF modulates intracellular signalling pathways that regulate endothelial cell division, migration, and survival. VEGF and its two receptors, Flt-1 and Flk-1/KDR are crucial regulators of physiological and pathological blood vessel growth[23].Invasive cyto trophoblasts express several angiogenic factors and receptors, including VEGF-A, PlGF, andVEGFR-1 (Flt1)[42]. Hence blocking these angiogenic factors results in the development of Preeclampsia.

sFlt-1 inhibits placental growth factor signalling and which is also a potent inhibitor of "vascular endothelial growth factor" which is raised in pregnant women with Preeclampsia[26]

During normal pregnancy, adequate oxygen and nutrient delivery to the developing utero placental unit occurs and remodel the maternal uterine spiral arteries. This process results in the conversion of the small-diameter spiral arteries, high-resistance into high-capacitance, low-resistance vessels[17].

**Placental formation during the early stage of pregnancy:**

sFlt1, Tie-1, Tie-2, (VEGFR-1), and VEGFR-2 are essential for normal placental vascular development. Any Alteration in these factors in gestation leads to inadequate cytotrophoblast invasion seen in the placentas of women with Preeclampsia[28].

During implantation, the uterine endometrium and inner third of myometrium is invaded by extra villous cytotrophoblasts. Invasive trophoblasts express maternal endothelial cell markers such asCD31, αvβ3 integrin, VCAM-1, and VE-cadherin (Zhou et al 1997[59], 2002[60]).

The markers of endothelial activation are von Willebrand antigen , Cellular fibronectin , Soluble tissue factor, Soluble E-selectin, Platelet-derived growth factor, and Endothelin which is altered in women with Preeclampsia.

**Endothelin**

Endothelin-1 (ET-1) has an important role in preeclampsia. ET-1 is a potent endothelium derived vaso constrictor. Endothelin-1 acts in the endothelin type A (ETA)receptor in the vascular smooth muscle. Many studies indicating that the elevated levels of plasma ET-1 seen in the preeclamptic group and also circulating ET-1 level correlates with disease symptoms[39].

Soluble fms-like tyrosine kinase (sFlt1) has increased expression seen with decreased expression of placental growth factor (PIGF) that impairs vascular endothelial growth factor (VEGF) signalling, were the first abnormalities generated due to placental hypoxia/ischaemia, these factors target the maternal vascular endothelium of women who expected to develop Preeclampsia ( Krauss et al. 1997[55], Gilbert et al. 2008[57]).

These studies suggest that angiogenic imbalance could be a reliable marker of Preeclampsia and it allows detection before the onset of symptoms, and also angiogenic factors may be better markers of intrauterine growth retardation regardless of the presence or absence of Preeclampsia[17.]

## Factors responsible for microvascular dysfunction and Hypertension

### Immune factors and inflammation

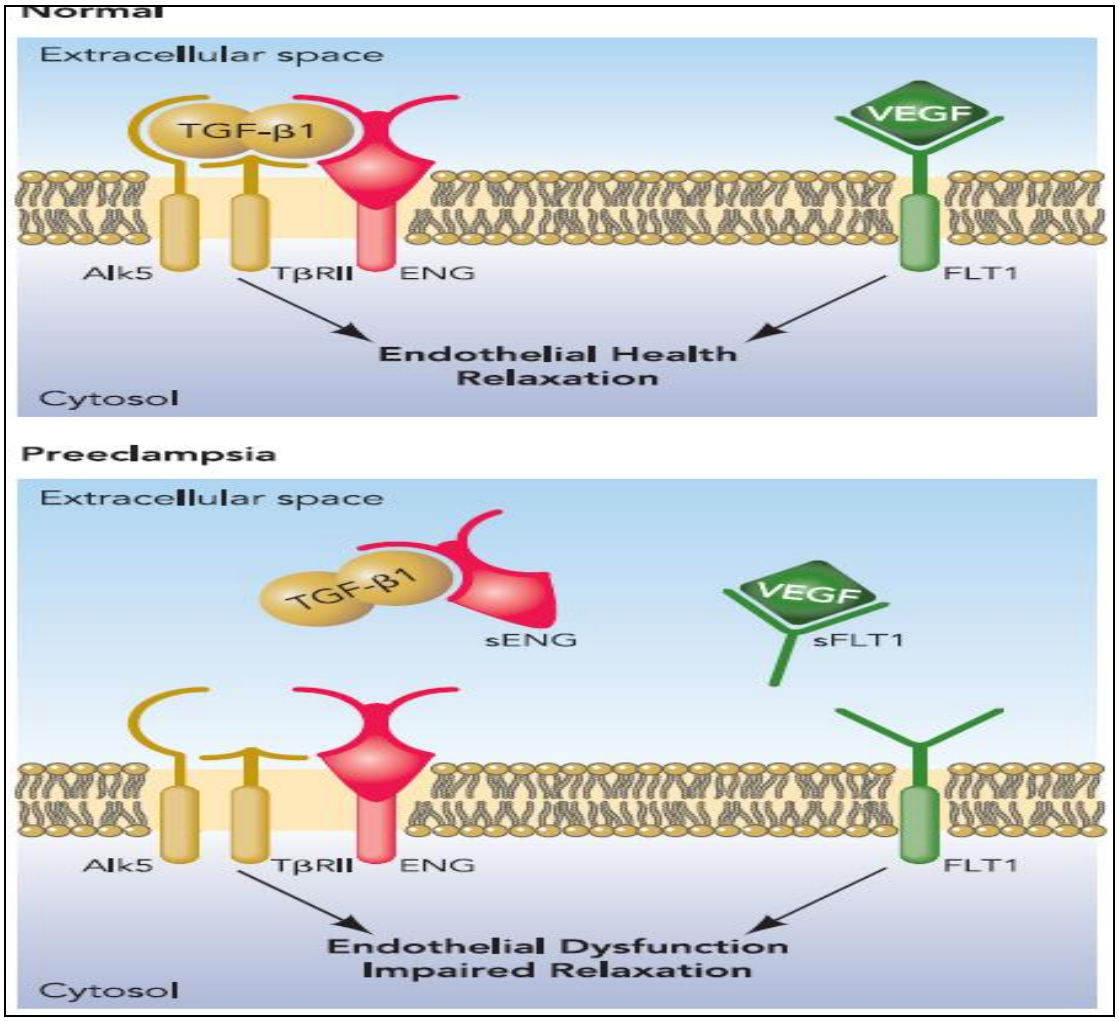
"Killer-cell immunoglobulin-like receptor" (KIR) family of uNK cells and HLA-C genotypes of trophoblasts were found as immunogenic background in unsuccessful pregnancies of Preeclampsia. It is found that both fetal HLA-C2 haplotype and maternal KIR-AA genotype are associated with increased risk of Preeclampsia as per hybi et all[65].

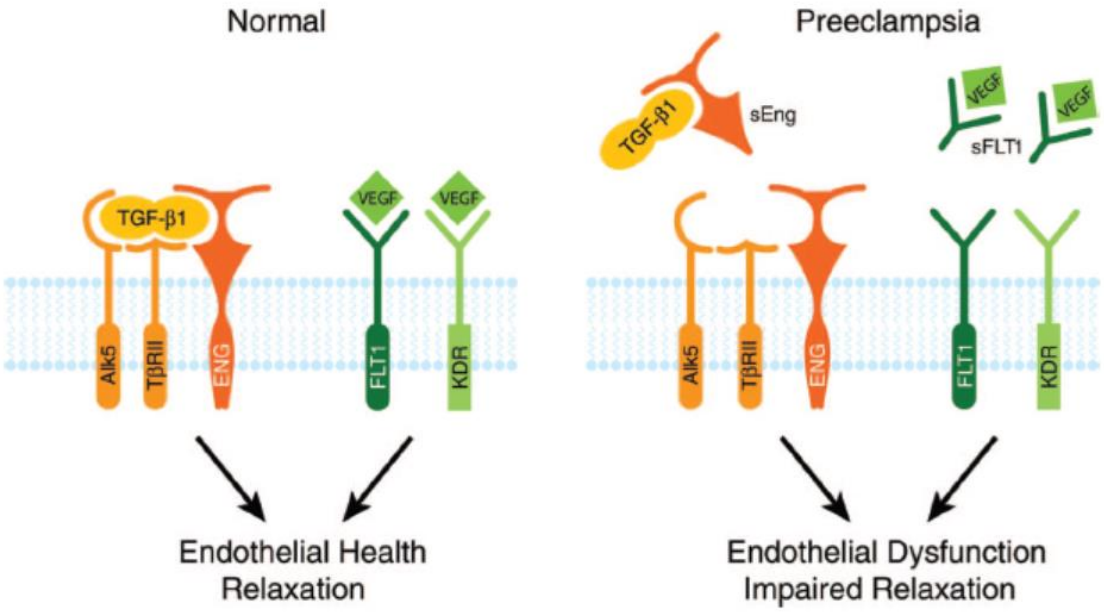
### Angiogenic factors

There is an imbalance between proangiogenic and antiangiogenic factors which is responsible for Preeclampsia. The important antiangiogenic factor is sFlt-1. sFlt-1 is a soluble splice variant of the VEGF receptor-1 and it prevents the actions of proangiogenic molecules, such as VEGF and PlGF in target tissues[17].

Molecular pathogenesis of Preeclampsia begins with the discovery of alterations in placental antiangiogenic factors. Hypoxia is an important regulator[28]. In response to placental hypoxia, antiangiogenic factors released which enter the maternal blood stream . These antiangiogenic factors, such as sFlt1 and soluble endogolin, produce systemic endothelial dysfunction, resulting in increase in blood pressure, proteinuria, and the other systemic manifestations of Preeclampsia. Exogenous administration of sFlt-1 has been shown to cause

## SOLUBLE FMS LIKE TYROSINE KINASE – I



increase in blood pressure, affects glomerular endothelium (endotheliosis, a pathological renal lesion seen in Preeclampsia) and produces proteinuria and in rats[66].

Pregnancy associated Plasma Protein-A (PAPP-A) levels reduced an antenatal maternal serum biomarker, could also be used as an early marker for pregnancies at-risk of Preeclampsia[67].

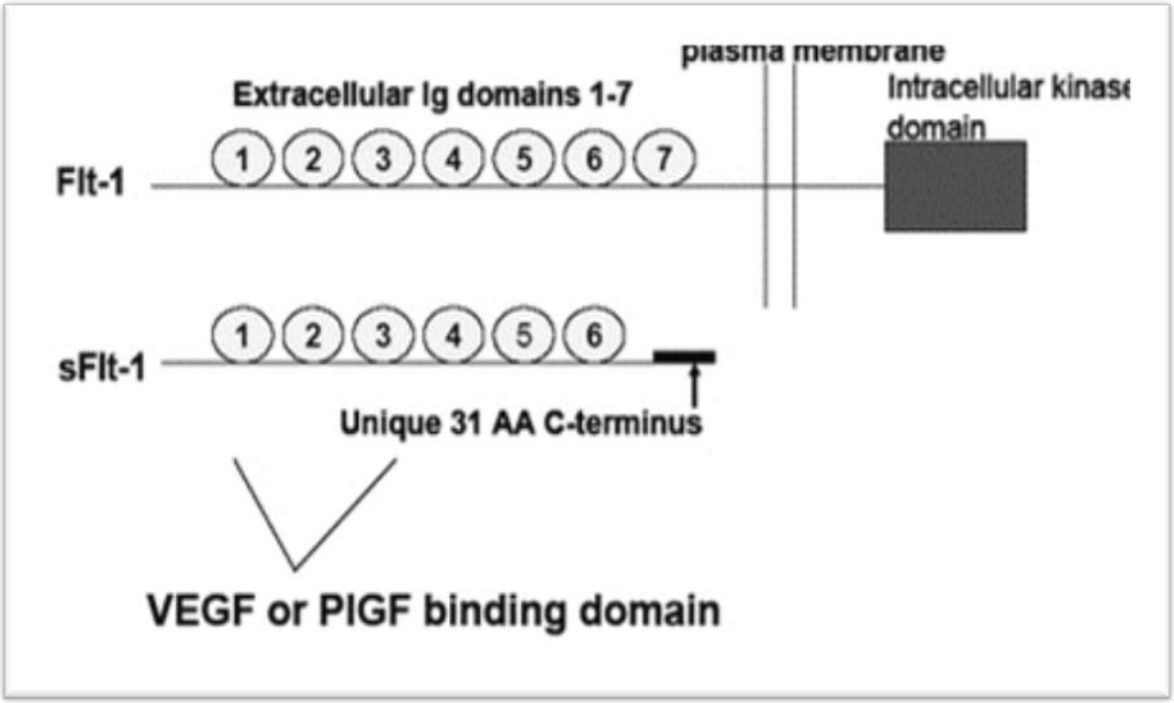
There is no appropriate specific screening test available till date which can predict the development of Preeclampsia. Serum concentrations of sFlt-1, PlGF and many other biomarkers of placental development have been suggested to have a predictive value to help to identify women at risk[41]. However, there are few trials which studied the utility of PlGF and sFlt-1 as biomarkers which can be used in both early diagnosis and in predicting who are those can develop Preeclampsia[52].

**"Soluble fms-like tyrosine kinase-1" (sflt-1)**

sFlt-1, a splice variant of the vascular endothelial growth factor (VEGF) receptor, lacking the cytoplasmic and transmembrane domains, acts as a potent PlGF and VEGF antagonist . This protein acts by adhering to the receptor-binding domains of placental growth factor (PlGF) and vascular endothelial growth factor (VEGF), preventing their interaction with endothelial receptors on the cell surface and thereby inducing endothelial dysfunction , thus

preventing their availability to stimulate angiogenesis and maintain endothelial integrity. sFlt-1 is produced by various tissues, including the troboblastic layer of placenta and circulating monocytes .

VEGF ligands and receptors are highly expressed by placental tissue in the first trimester. In Preeclampsia, there is shallow trophoblast invasion and inadequate vascular remodeling leading to a relatively hypoxic environment and an increase in sFlt-1 production . This increase in circulating levels of sFlt-1 reduce the circulating levels of VEGF and promote endothelial cell dysfunction.




FLT1 gene encodes a member of the vascular endothelial growth factor receptor (VEGFR) family. VEGFR family members are having receptor tyrosine kinases (RTKs) which contain an extracellular ligand-binding region with seven

immunoglobulin (Ig)-like domains, a transmembrane segment, and a tyrosine kinase (TK) domain within the cytoplasmic domain.

The role of VEGFR1 has generally been characterised as a decoy receptor. It has been hypothesised that high levels of sVEGF-R1 antagonise the effects of VEGF and PlGF on placental development, vascularisation and maternal endothelial cell function, and thus the increase in sVEGFR1 in maternal plasma has been postulated to inhibit VEGF-A. However, VEGF-A induces increased vascular permeability, vasodilatation and angiogenesis.

Increased sVEGFR1 should therefore prevent the permeability responses of Preeclamptic plasma, not induce them. Thus increased sVEGFR1 acting on VEGF-A by itself does not explain the symptoms of preeclampsia or experimental findings of the effect of Preeclamptic plasma, but sVEGFR1 acting on PlGF, and removing the inhibition of VEGF would explain the symptoms.

Soluble fms-like tyrosine kinase-1 binds to VEGFR-1, VEGFR-2 and placental growth factor plays an important role in angiogenesis and vasculogenesis. Increased sVEGFR-1 levels were seen in the placenta, plasma and serum of preeclamptic women.

The magnitude of the imbalance between angiogenic (VEGF) and antiangiogenic factor (sVEGFR-1) concentrations in maternal blood is greater in early-onset (severe) than in late-onset (mild) Preeclampsia [23].

## THE VASCULAR ENDOTHELIAL GROWTH FACTOR FAMILY

Vascular endothelial growth factor was first isolated in ascitic fluid. It was first termed as Vascular Permeability Factor in 1983 due its ability to increase vascular permeability .It promotes endothelial cell proliferation.

## THE VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTORS

Vascular endothelial growth factor receptor-1 (VEGFR-1) (or fms-like tyrosine kinase-1 (flt-1)) andVEGFR-2 (kinase insert domain-containing receptor or fetal liverkinase-1)[23]arethe vascular endothelial growth factor receptors. This family contains five members in humans, VEGF-A, -B, -C, and -D and Placental Growth Factor (PlGF). The most widely studied form is VEGF-A (or simply VEGF), is expressed as numerous isoforms secondary to alternative exon splicing resulting in mature proteins varying from 121 to 206 amino acids[18].

It exerts its biological effects through two high-affinity tyrosine kinase receptors that is, VEGFR-1 andVEGFR-2. VEGFR-1 has 10-fold higher affinity than VEGFR-2 for VEGF binding, but VEGFR-2 has a more important functional role in mediating signalling events involved in endothelial cell mitogenesis, migration, survival and vascular permeability[23]. sFlt-1 is a circulating soluble receptor for both VEGF and PlGF, which when increased in maternal plasma leads to less circulating free VEGF and free PlGF,

## VEGF and VEGFR expression in Preeclampsia

### VEGF

Abnormally high levels of VEGF in Preeclamptic plasma and serum amniotic fluid, umbilical cord serum and urine have been described using radioimmunoassay or competitive enzyme immunoassay

VEGF is a well-known promoter of angiogenesis; it also induces nitric oxide and vasodilatory prostacyclins in endothelial cells, suggesting a role in decreasing vascular tone and blood pressure implicated in glomerular healing, and anti-VEGF compounds in addition to its role in Preeclampsia it also increase apoptosis, impair glomerular capillary repair, and increase proteinuria in patients with mesangioproliferative nephritis .

## Placental Growth Factor

PlGF is a 45-to 50-kd dimeric glycoprotein with 53% sequence identity to VEGF. It has PlGF- 1 and PlGF-2 protein isoforms. The decrease in PlGF production is the reason for abnormalities of placental angiogenesis which man be through effects on other vascular growth factors. Decreased concentrations of circulating free PlGF and free VEGF have been noted during clinical Preeclampsia and even before its onset. Many studies, say that PlGF is reduced in women with Preeclampsia[53,54].

The reason for low levels of PIGF was found to be due to its binding with sFlt-1 rather than reduced production by the Preeclamptic placenta.

**Endogolin**

Soluble Endogolin (sEng) plays an important role in the pathophysiology of Preeclampsia. Endogolin is a pro-angiogenic factor that protects endothelial cells under hypoxia and regulate NO (nitric oxide) dependent vasodilatation **.**

Preeclamptic placentas overexpress sFlt1 and sEng mRNAs as well as sFlt1and sEng proteins. On the other hand, Soluble Endogolin (sEng) is an anti-angiogenic protein. It consists of the extra cellular part of the molecule that may be produced through the proteo-cleavage of the placental membrane-bound form. It decreases endothelial nitric oxide by inhibiting TGF signalling and this leads to endothelial dysfunction.

In vitro, Soluble Endogolin acts as a negative regulator of angiogenesis by competitive interaction with TGF, thereby impairing capillary formation by endothelial cells.

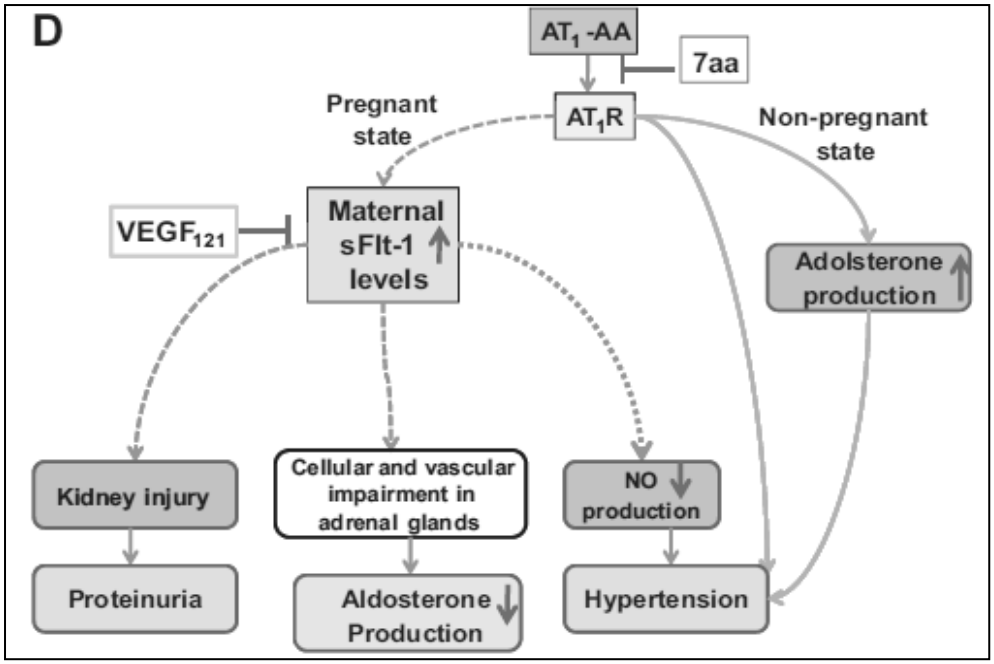
Furthermore, it induces high arterial pressure and vascular permeability in pregnant rats in which the protein was over-expressed. Very interestingly, the introduction of Endogolin in the pregnant animals induced renal, placental and hepatic changes reminiscent of the HELLP syndrome .

Soluble endogolin could be used as a biochemical marker for prediction of PE in the second trimester, as it is usually increased 10-12 weeks before the clinical symptoms of the disease manifest itself.

Soluble endogolin is present in substantial excess in Preeclampsia, patients compared to normotensive controls however whether this factor is specific for Preeclampsia or it is also increased with other hypertensive diseases of pregnancy such as gestational or chronic hyper-tension is the aim of future studies which are needed to clarify this issue.

The concentration of Soluble Endogolin appear to increase with the severity of Preeclampsia, especially in Preeclampsia, complicated by HELLP syndrome and IUGR. However, prediction of cut-off value for Preeclampsia, and gestational hypertension was not attempted due to the non-consistent results of studies assessing Soluble Endogolin level.

Some of these studies showed variable Soluble Endogolin level in pregnancies complicated by Preeclampsia, different factors affecting Soluble Endogolin level either related to the race or the BMI are still investigated so this point is still a point of discussion and needs further studies.




This signalling pathway shows elevated circulating and placental levels of the soluble form of the VEGF receptor, fms-like tyrosine kinases (sFlt)-1.

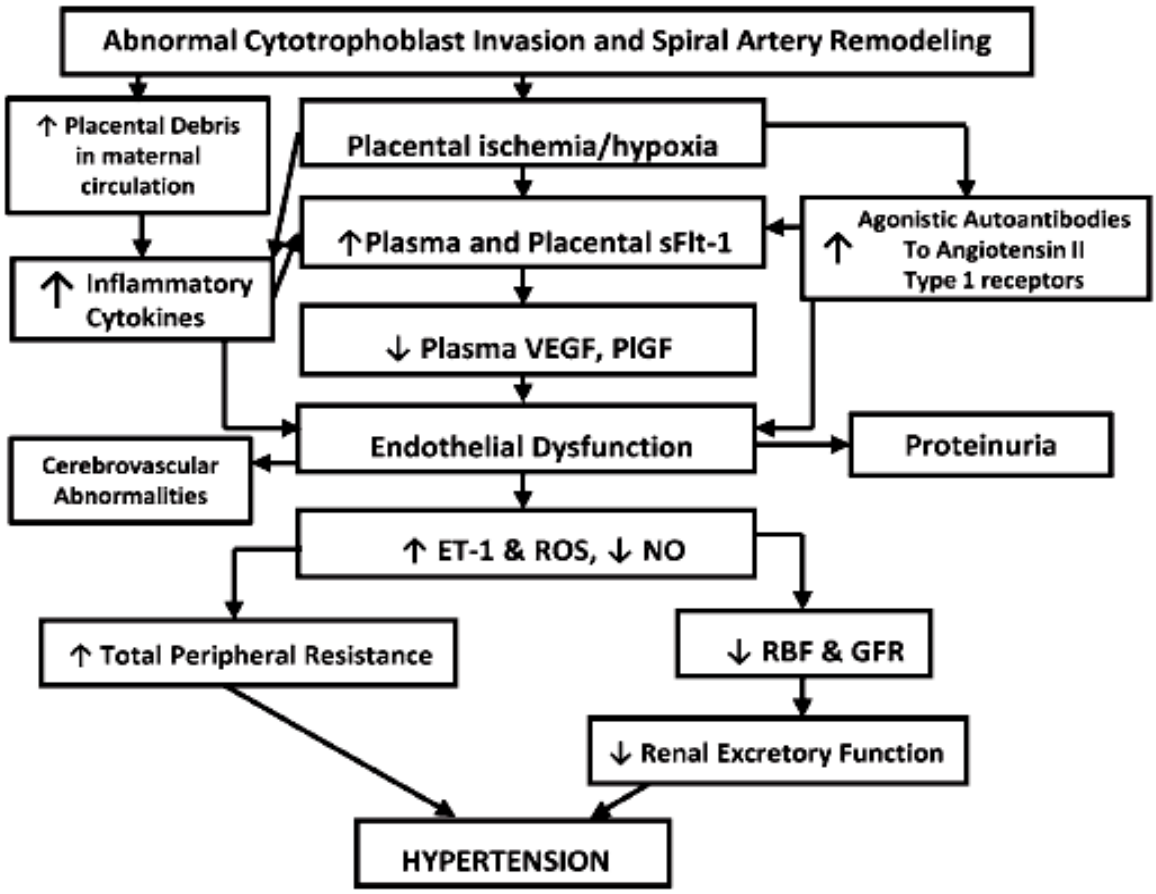
**Nitric oxide**

VEGF is a proangiogenic factor that stimulates NO production and endothelial NO synthase (NOS) protein levels[40]. NO production is elevated in normal pregnancy and play an important role in the vasodilatation.

Sandrim et al[69] study indicates that women with Preeclampsia have reduced plasma and whole blood nitrite levels, which negatively correlate with the potent VEGF inhibitor sFlt-1.

Facemire et al[70] demonstrated that inhibition of VEGF receptor type 2 (VEGFR2) results in hypertension through impaired NO synthesis and

contributes to the enhanced ET-1 production and elevations in blood pressure in response to sFlt-1-induced hypertension in pregnant rats[39].




In view of diagnosing the condition , the development of soluble angiogenic markers discussed above help in the screening and early diagnosis of Preeclampsia.

The purpose of this study is to use sFlt-1 as a predictive marker of Preeclampsia in early trimester.

# MATERIALS AND METHODS

# MATERIALS AND METHODS

The study was carried out in a tertiary care centre, Raja Mirasudar Hospital, Thanjavur, attached to our Medical College. 90 primigravida were included in the study.

The diagnosis of gestational hypertension was done as per the norms of American college of Obstetrics and Gynecologists.

All the participants were inquired by a questionnaire

1) Name of the person
2) Age of the person
3) Address
4) Complaints
5) Last Menstrual period
6) Head ache
7) Inability to tolerate Bright light, Blurred vision
8) Double vision
9) Nausea, Vomiting
10) Right sided upper Quadrant pain
11) Epigastric pain
12) Dyspnoea
13) Oliguria

14) Bleeding tendency

15) Sudden weight gain

16) Edema

17) Tiredness

**FAMILY HISTORY**

1) Preeclampsia

2) Twins

3) Hypertension

4) Diabetic

**DRUG HISTORY**:

1) Thiazides

2) Retinoid

3) Anti retro viral therapy

4) Estrogen

5) β-blockers

6) Progestin

7) Glucocorticoids

**INCLUSION CRITERIA:**

1) Age between 20-45

2) Primi Gravida

3) 16-20 Weeks of pregnancy

**EXCLUSION CRITERIA**

1) Known Diabetic, Hypertensive

3) Edema

4) Proteinuria, oliguria

5) Hepatic disease

6) Involvement of other Organs.

Clinical examination of participants were carried out to rule out Diabetes, Hypertension, edema, proteinuria, oliguria, Hepatic disease, involvement of other Organs.

# METHODOLOGY

## BLOOD COLLECTION:-

Informed consent was obtained for each primigravida prior to the study. 5ml of blood samples were collected by vene puncture under strict aseptic precaution as per the inclusion criteria. The samples were centrifuged and serum separated. One part of the sample was taken and analysis of ALT,AST, Creatinine & Platelet count were done immediately. Remaining part of the sample was stored for analysis of at - 70º C. Enzyme-linked immunosorbent assays (ELISA) for sFlt-1was performed with commercially-available kits.

In sterile tubes urine samples were collected and tested for protein with dipstick method.

## ANALYSIS OF BLOOD SAMPLES:

The serum collected above was used for the estimation of the following parameters

## ESTIMATED PARAMETERS:

1. Soluble fms like tyrosine kinase 1 by Elisa method
2. Serum Aspartate Transaminase - Modified IFCC Method.
3. Serum Alanine Transaminase - Modified IFCC Method.
4. Serum Creatinine - Modified Jaffe's Method
5. Platelet count - cell counter
6. Urine protein – dipstick method

7. Blood pressure measured by sphygmomanometer
8. weight

Gestational Hypertension - defined as diastolic blood pressure ≥ 90mmHg and systolic blood pressure ≥ 140mmHg in normotensive patients after 20 weeks of pregnancy at least two consecutive measurements 6 hrs apart urine protein measured by dipstick method on a random urine sample.

**ESTIMATION OF "SOLUBLE FMS LIKE TYROSINE KINASE 1"**

**METHODOLOGY :**

Enzyme-linked immune sorbent assay (ELISA)

**PRINCIPLE :**

The basis of ELISA used by this kit is Biotin double antibody sandwich technology to assay human "soluble fms like tyrosine kinase 1". Samples & calibrators containing "soluble fms like tyrosine kinase 1" were added to the wells that are pre-coated with an anti humans VEGF –R1 antibody and then incubated. Then biotin-labelled anti human sVEGF –R1antibody was added which unites with streptavidin-HRP forming immune complexes. The unbound enzymes were removed after incubation by washing. TMB Substrate was added which changes the colour of the solution to blue and then yellow by the effect of acid. The shades of solution positively correlate with the concentration of human sVEGF –R1antibody.

**MATERIALS SUPPLIED IN THE KIT:**

1. Human sVEGF –R1 Standard lyophilised, 1000pg/ml upon reconstitution
2. Standard dilution - 3ml
3. Mono clonal antibody to Human sVEGF – R1Coated ELISA plate
4. Streptavidin-HRP - 6ml, Conjugate diluent (20ml)
5. Washing concentration (30X) - 20ml
6. Assay buffer concentration (20X) 5ml
7. Human sVEGF –R1 antibodies labelled with biotin - 1ml
8. Stop solution - 6ml (1M Phosphoric acid)

Other materials required were

1. 37°C incubator
2. Precision pipettes and disposable pipette tips
3. Disposable tubes for sample dilution
4. Standard enzyme reader
5. Distilled water
6. Adsorbent paper

**PREPARATION OF REAGENTS**

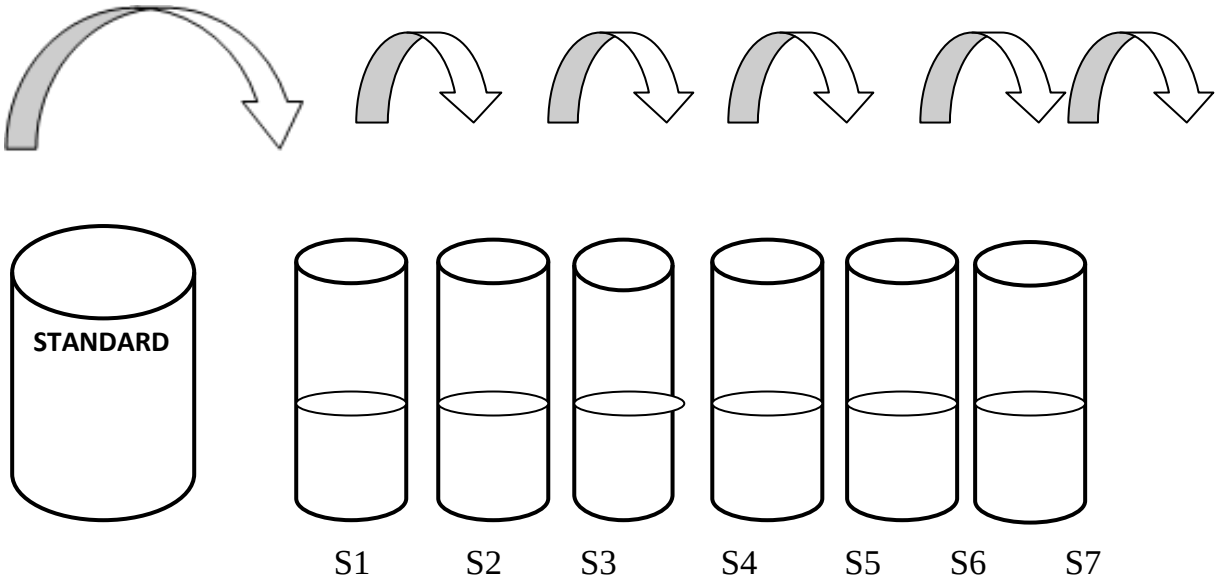
All samples and reagents were brought to room temperature which takes about 30 minutes. Specimens were mixed thoroughly by gentle inversion and visible particulate matter was cleared by low speed centrifugation.

- Wash buffer: 20x wash solution was diluted by pouring the total contents of the bottle (50ml) into a 1L graduated cylinder and 950ml of deionised water was added to make a final volume of 1000ml. It was then thoroughly mixed.

- Assay buffer: 20x assay buffer solution was diluted by pouring the total contents of the bottle (5ml) into a 100ml graduated cylinder and 95ml of deionised water was added to make a final volume of 100ml. It was then thoroughly mixed.
- Dilution of standard solution : The kit had a standard of original concentration which was supposed to be diluted in small tubes by following the instructions:

Seven tubes one for each standard pointS1,S2,S3,S4,S5,S6,S7 was labelled. 1:2 serial dilutions were prepared.

225 µl of assay buffer pipetted into each tube. 225µl of reconstituted standard pipetted into the first tube, labelled S1,and mixed. 225 µl of reconstituted standard pipetted into the second tube, labelled S2,and mixed. 225 µl of reconstituted standard pipetted into the third tube, labelled S3,and mixed. 225 µl of reconstituted standard pipetted into the fourth tube, labelled S4,and mixed. 225 µl of reconstituted standard pipetted into the fifth tube, labelled S5,and mixed.225 µl of reconstituted standard pipetted into the six tube, labelled S6,and mixed. 225 µl of reconstituted standard pipetted into the seven tube, labelled S7,and mixed. 225 µl fromS7 labelled tube pipetted & Discarded.




- Streptavidin-HRP : ready to use
- Biotin labelled Human sVEGF – R1antibodies : ready to use
- Stop solution : ready to use

**ASSAY PROCEDURE**

The number of stripes needed was decided by the number of samples to be tested and by that of standards.

1. Blank well: no sample, 100 µl assay buffer was added to the blank well.
2. Standard solution well: 50µl standard and 50µl assay buffer were added.
3. Sample well: 50µl sample, 50µl assay buffer were added.
4. 50µl of biotin conjugate was added to all wells

The plate was shaken gently to mix them up and then it was incubated at 25°C for 120 minutes. Then the ELISA plate was covered with a seal plate membrane.

**Washing:**

The ELISA plate was covered by a seal membrane ,which the was carefully removed and drain the liquid was drained . Each well was filled with 350µl wash solution. After standing for 30 seconds, the liquid was drained. To blot the ELISA plate, it was pat hard on bibulous papers on the test bed, several times downward. The procedure was repeated 6 times.

4. 100 µl of Streptavidin- HRP conjugate to all wells. Then the ELISA plate was covered with a seal plate membrane. They were mixed by gentle shaking. Incubation was done for 60 minutes at 25°C away from light.

**Washing :**

The ELISA plate was covered by a seal membrane, which the was carefully removed and the liquid was drained. Each well was filled with 350µl wash solution. After standing for 30 seconds, the liquid was drained. To blot the ELISA plate, it was pat hard on bibulous papers on the test bed, several times downward. The procedure was repeated 6 times.

100 µl of TMB Substrate solution was added to all wells.

Incubated at 25°C for 30 minutes.

Direct exposure to intense light was avoided.

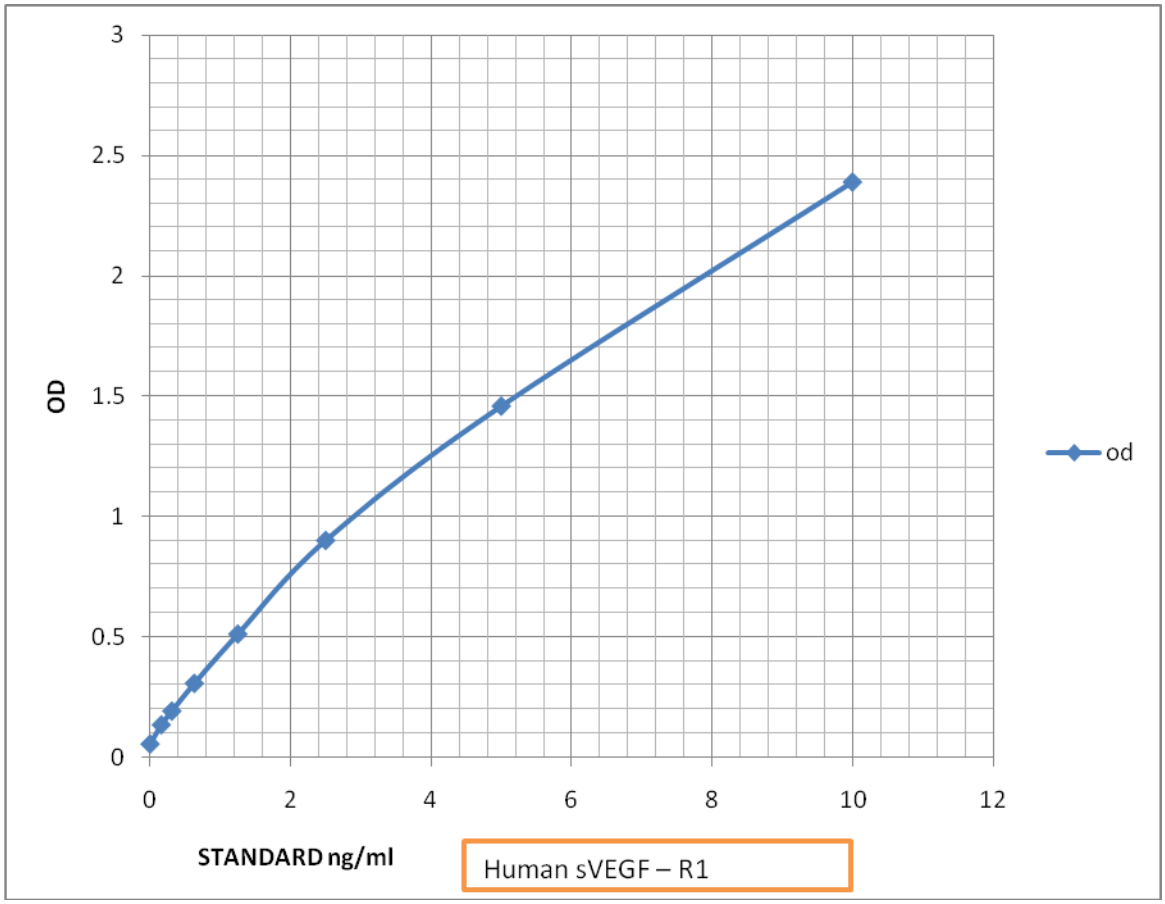
- Stop : To stop the solution 50µl stop solution was added to each well. At this moment, there was an immediate change in colour from blue to yellow.

- Assay : Taking blank as zero, the absorbance (OD) of each well was measured at 450nm wavelength within 10 minutes after adding the stop solution.

- According to the concentration of standards and their corresponding OD values, the linear regression equation of standard curve was calculated. Then the concentration of the sample was calculated according to their corresponding OD values.

**CALCULATION OF RESULTS**

Calibration graph : The standard curve was constructed by plotting concentration of each Human sVEGF – R1 in ng/ml along the x-axis against the OD values of the corresponding calibrator along y-axis.




**Human sVEGF – R1 values of samples :**

The Human sVEGF – R1 concentration of each sample was found by locating the point on the curve corresponding to the absorbance values for the samples and reading its corresponding concentration in ng/ml from the x-axis.

**ASSAY RANGE** : 45pg/ml to 1500pg/ml

**SENSITIVITY :** 0.1pg/ml

**VALIDITY&STORAGE :**

serum samples were 6 months when stored at 2-8°C and 12months at -20°C.

**QUANTITATIVE ESTIMATION OF SERUM CREATININE**

**MODIFIED JAFFE'S REACTION, INITIAL RATE**

## PRINCIPLE OF THE METHOD

Creatinine reacts with picric acid in an alkaline medium to form an orange-yellow colour which is termed as Jaffe's reaction. The initial rate method is introduced to improve the specificity of the test. The optical density of the orange-yellow colour formed is directly proportional to the concentration of creatinine, which is measured photometrically at 500 to 520nm.

## REAGENT COMPOSITION

- Reagent 1 - Picric Acid

  25.8 mmol/L

- Reagent 2 - Sodium Hydroxide

  95 mmol/L

- Creatinine standard : 2 mg/dl or (0.166 mmol/L)

## REAGENT PREPARATION

Equal volumes of Reagent 1 and Reagent 2 are mixed and wait for 15 minutes before use.

## STORAGE AND STABILITY

Reagents 1, 2 and standard when unopened remain stable till the expiry date. The Working Reagent is stable for 21 days at 2-8°C. The absorbance of the reagent blank should be <0.3 at 505nm when read against distilled water.

**ASSAY PARAMETERS**

Wavelength (nm) : 505

Mode : fixed time

Sample volume : 100 µl

Reagent volume : 1000 µl

Lag time : 20 sec

Kinetic interval : 60 sec

No. of readings : 1

Reaction temperature : 37°C

Normal low : 0.6 mg/dl

Normal high : 1.4 mg/dl

Reaction direction : increasing

Linearity low : 0 mg/dl

Linearity high : 25 mg/dl

Absorbance limit (max) : 0.3

Standard concentration: 2 mg/dl

Blank with : water

Units : mg/dl

## ASSAY PROCEDURE

| PIPETTE | STANDARD | TEST |
|---|---|---|
| Working reagent | 1000µl | 1000µl |
| Standard | 100µl | - |
| Test | - | 100µl |

Mixed well and read the initial absorbance within 20 seconds of mixing and final absorbance at 80 seconds.

## CALCULATION

The results are calculated as follows :

$\Delta A = A_2 - A_1$

$$\text{Creatinine concentration in mg/dl} = \frac{\Delta A \text{ of the test}}{\Delta A \text{ of standard}} \times \text{concentration of standard(mg/dl)}$$

## LINEARITY

The assay is linear upto a value of 25mg/dl. For higher values the samples were diluted with normal saline and the assay repeated. Then the results were multiplied with the dilution factor.

**NORMAL VALUES**

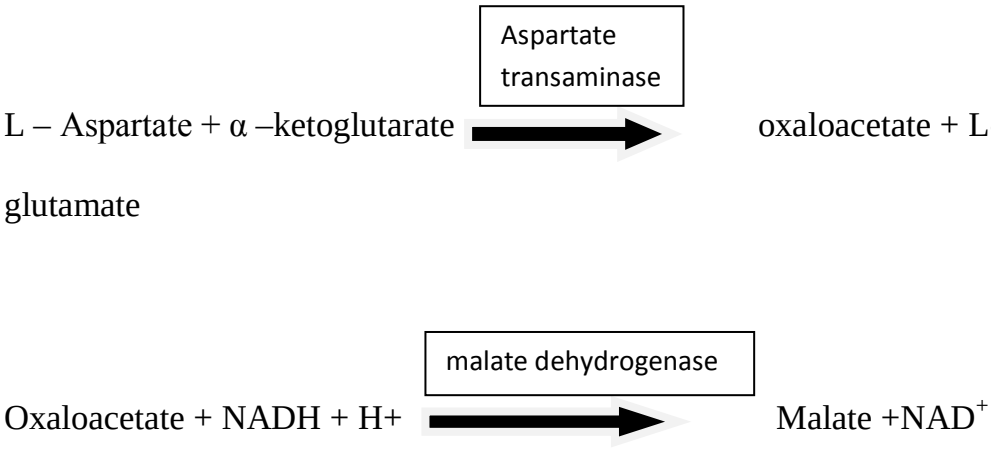
For males : 0.7 - 1.4 mg/dl

For females : 0.6 - 1.2 mg/dl

**ESTIMATION OF ASPARTATE TRANSAMINASE: (AST)**

**METHOD: MODIFIED IFCC METHOD**

**PRINCIPLE:** Aspartate transaminase (AST) catalyses the transfer of amino group from L-aspartate to α –ketoglutarate to yield oxaloacetate and L-glutamate. The oxaloacetate undergoes reduction with simultaneous oxidation of NADH to NAD in the malate dehydrogenase catalysed indicator reaction. The resulting rate of decrease in absorbance at 340nm is directly proportional to the AST activity.




**REAGENT:1**

Tris buffer (pH 7.8) 20mmol/L

L-Aspartate 230mmol/L

LDH >33.3µkat/L

2-Oxaloacetate 13.21mmol/L

MDH >33.3µkat/L

**REAGENT: 2**

NADH 1.51 mmol/L

**REAGENT PREPARATION:**

Working reagent was prepared by mixing 4 parts of R1 with 1 part of R2 per assay tube.

**PROCEDURE:**

500 µl of working reagent was mixed with 25µl of sample , mixed well and aspirated.

**CALCULATION:**

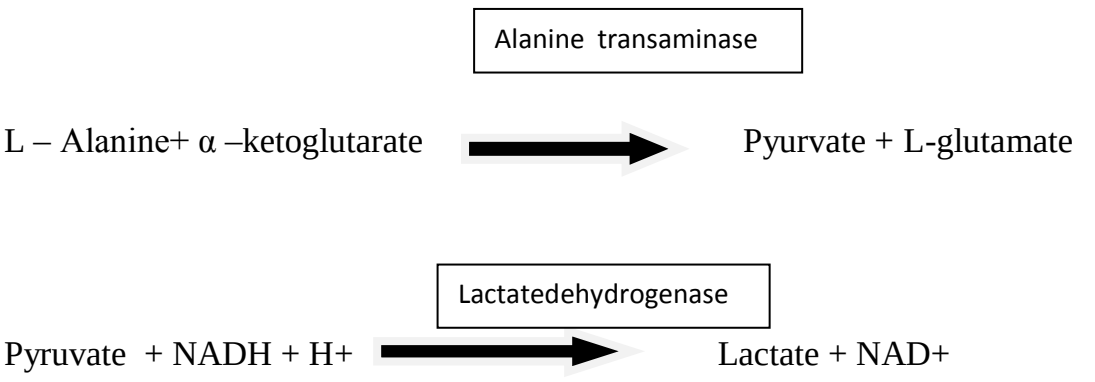
SGOT (AST) activity (IU/L) = DA/ min x factor (3376)

**NORMAL RANGE:** 10 – 40 U/L

**ESTIMATION OF ALANINE TRANSAMINASE: (ALT)**

**METHOD: MODIFIED IFCC METHOD**

**PRINCIPLE:** Alanine transaminase (ALT) catalyses the transfer of amino group from L-alanine to α –ketoglutarate to yield pyruvate and L- glutamate. Pyruvate in the presence of NADH and lactate dehydrogenase is reduced to L-lactate. In this reaction NADH is oxidized to NAD.




**REAGENT:1**

Tris buffer (pH 7.8) 100mmol/L

L-Alanine 230mmol/L

LDH > 4U/ml

α- ketoglutarate 13.20mmol/L

**REAGENT: 2**

β NADH 1.52 mmol/L

**REAGENT PREPARATION:**

Working reagent was prepared by mixing 4 parts of R1 with 1 part of R2 per assay tube.

**PROCEDURE:**

500 µl of working reagent was mixed with 25µl of sample , mixed well and aspirated.

**CALCULATION:**

SGPT(ALT) activity (IU/L) = $\triangle$ A/ min x factor (3376)

**NORMAL RANGE:**

For women: up to 32 U/L

For men : up to 65U/L

# RESULTS
# &
# STATISTICS

## RESULTS & STATISTICS

Our study is population based cohort study. The data were collected prospectively during pregnancy. In addition, the study was restricted to primigravida , The incidence and risk factors for Preeclampsia and gestational hypertension are increased with primigravida. Preeclampsia can be predicted by measuring serum level of sFlt-1 at a stage when the syndrome is clinically unrecognizable. In our study , primigravidae were selected, since nulliparity itself is a risk factor for the development of Preeclampsia.

Previous studies have shown that nulliparity increases the risk of developing Preeclampsia up to three-fold. Women who had any other known risk factors for developing Preeclampsia were excluded from the study. Smokers were also not included in the study because this has been shown to alter the sFlt-1 levels.

**Table 1 Age distribution of the study population (n=90)**

| Age group | Frequency | Percent |
|---|---|---|
| 21- 25 years | 52 | 57.8 |
| 25 - 30 years | 38 | 42.2 |
| Total | 90 | 100.0 |

Mean age: 24.92 years. Standard deviation: 2.309 years

Maximum: 30 years

Minimum: 21 years

**Figure 1:Pie chart showing age distribution of the study population (n=90)**

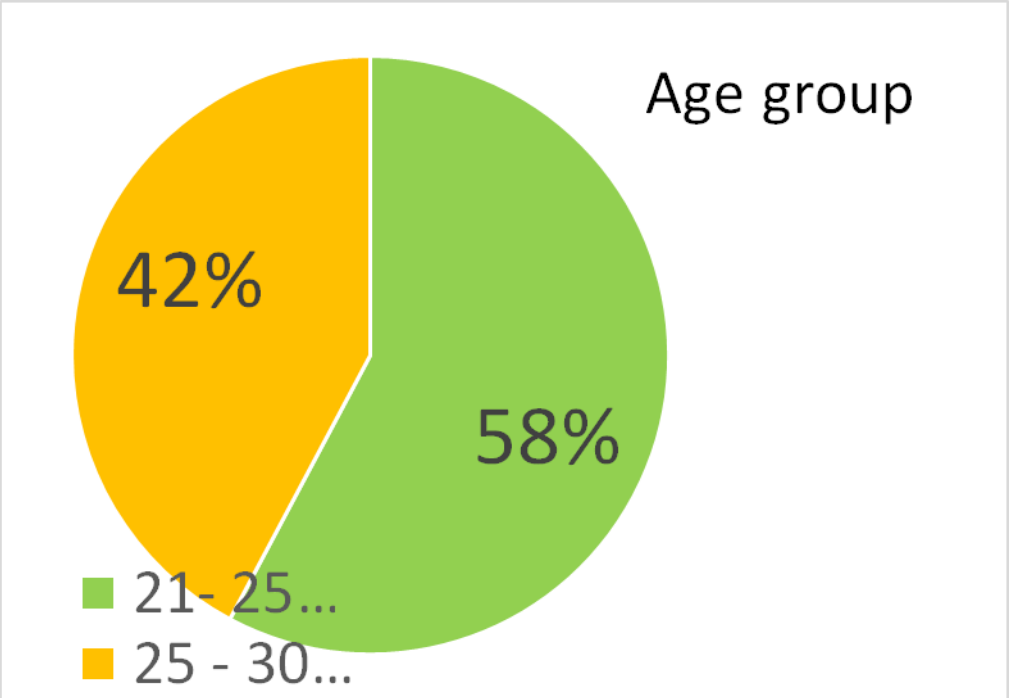


**Table 2 Distribution of the mean maternal weight in kg during various antenatal periods (n=90)**

| Statistic | Mean maternal weight in kg | | | | |
|---|---|---|---|---|---|
| | **17 to 20 weeks** | **21 to 28 weeks** | **29 to 36 weeks** | **36 to 40 weeks** | **Weight Gain during pregnancy** |
| Mean | 59.67 | 60.94 | 65.94 | 71.98 | 12.31 |
| Std. Deviation | 4.490 | 4.171 | 4.090 | 3.828 | 2.048 |
| Minimum | 45 | 47 | 49 | 55 | 8 |
| Maximum | 67 | 66 | 73 | 79 | 18 |

The mean weight gain during pregnancy of the study population was 12.3 kg

**Figure 2:Line diagram showing mean maternal weight in kg during various antenatal periods (n=90)**

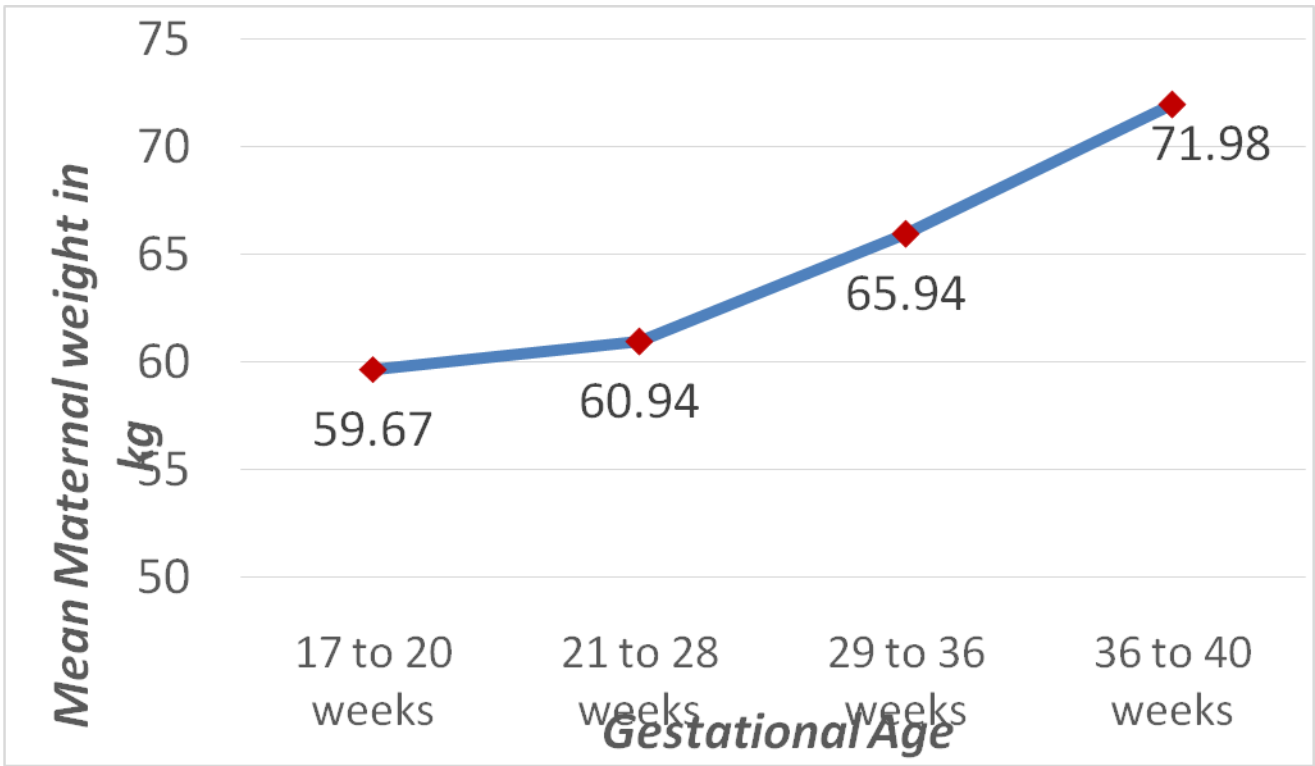


**Table 3 Distribution of the Mean maternal blood pressure during various antenatal periods (n=90)**

| Systolic Blood pressure | Mean | Standard Deviation | Minimum | Maximum |
|---|---|---|---|---|
| 17 to 20 weeks | 117.60 | 6.97 | 102.00 | 132.00 |
| 21 to 28 weeks | 106.80 | 14.25 | 90.00 | 156.00 |
| 29 to 36 weeks | 116.38 | 13.90 | 90.00 | 160.00 |
| 36 to 40 weeks | 120.96 | 15.37 | 102.00 | 156.00 |
| Difference between 1st and 4th reading | 3.36 | 12.23 | -16.00 | 40.00 |
| Post-natal period | 117.02 | 8.50 | 102.00 | 136.00 |

| Diastolic Blood pressure | Mean | Standard Deviation | Minimum | Maximum |
|---|---|---|---|---|
| 17 to 20 weeks | 82.34 | 3.80 | 76.00 | 89.00 |
| 21 to 28 weeks | 74.89 | 11.31 | 60.00 | 104.00 |
| 29 to 36 weeks | 82.89 | 8.68 | 70.00 | 110.00 |
| 36 to 40 weeks | 84.62 | 10.78 | 76.00 | 110.00 |
| Difference between 1st and 4th reading | 2.28 | 9.70 | -9.00 | 34.00 |
| Post-natal period | 80.89 | 5.69 | 60.00 | 90.00 |

Mean systolic pressure raised by 3 mmHg and mean diastolic pressure raised by 2 mmHg during pregnancy.

**Figure 3:Line diagram showing Mean maternal blood pressure during various antenatal periods (n=90)**

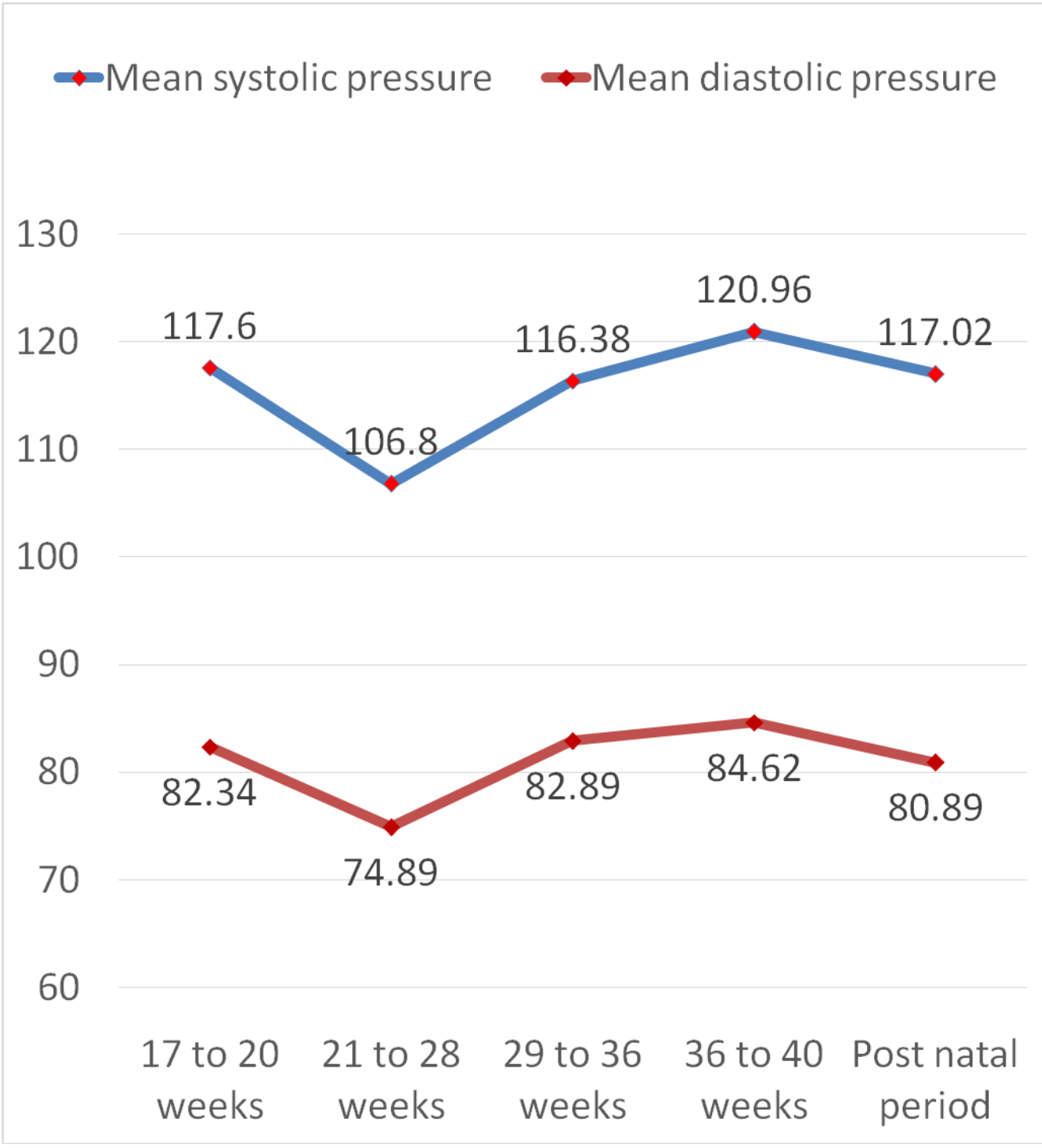


**Table 4 Distribution of the Mean maternal levels of transaminases during various antenatal periods (n=90)**

| ALT | Mean | Standard Deviation | Minimum | Maximum |
|---|---|---|---|---|
| 17 to 20 weeks | 26.79 | 3.78 | 22 | 38 |
| 21 to 28 weeks | 29.31 | 5.06 | 23 | 47 |
| 29 to 36 weeks | 28.97 | 10.13 | 22 | 68 |
| 36 to 40 weeks | 29.71 | 10.13 | 23 | 70 |
| Difference between 1st and 4th reading | 2.92 | 8.11 | -6.00 | 37.00 |
| **AST** | **Mean** | **Standard Deviation** | **Minimum** | **Maximum** |
| 17 to 20 weeks | 27.33 | 3.75 | 22 | 38 |
| 21 to 28 weeks | 28.60 | 6.21 | 23 | 53 |
| 29 to 36 weeks | 29.12 | 10.06 | 22 | 69 |
| 36 to 40 weeks | 29.33 | 9.92 | 22 | 68 |
| Difference between 1st and 4th reading | 2.00 | 7.73 | -10.00 | 34.00 |

**Figure 4:Line diagram showing Mean maternal transaminase levels during various antenatal periods (n=90)**

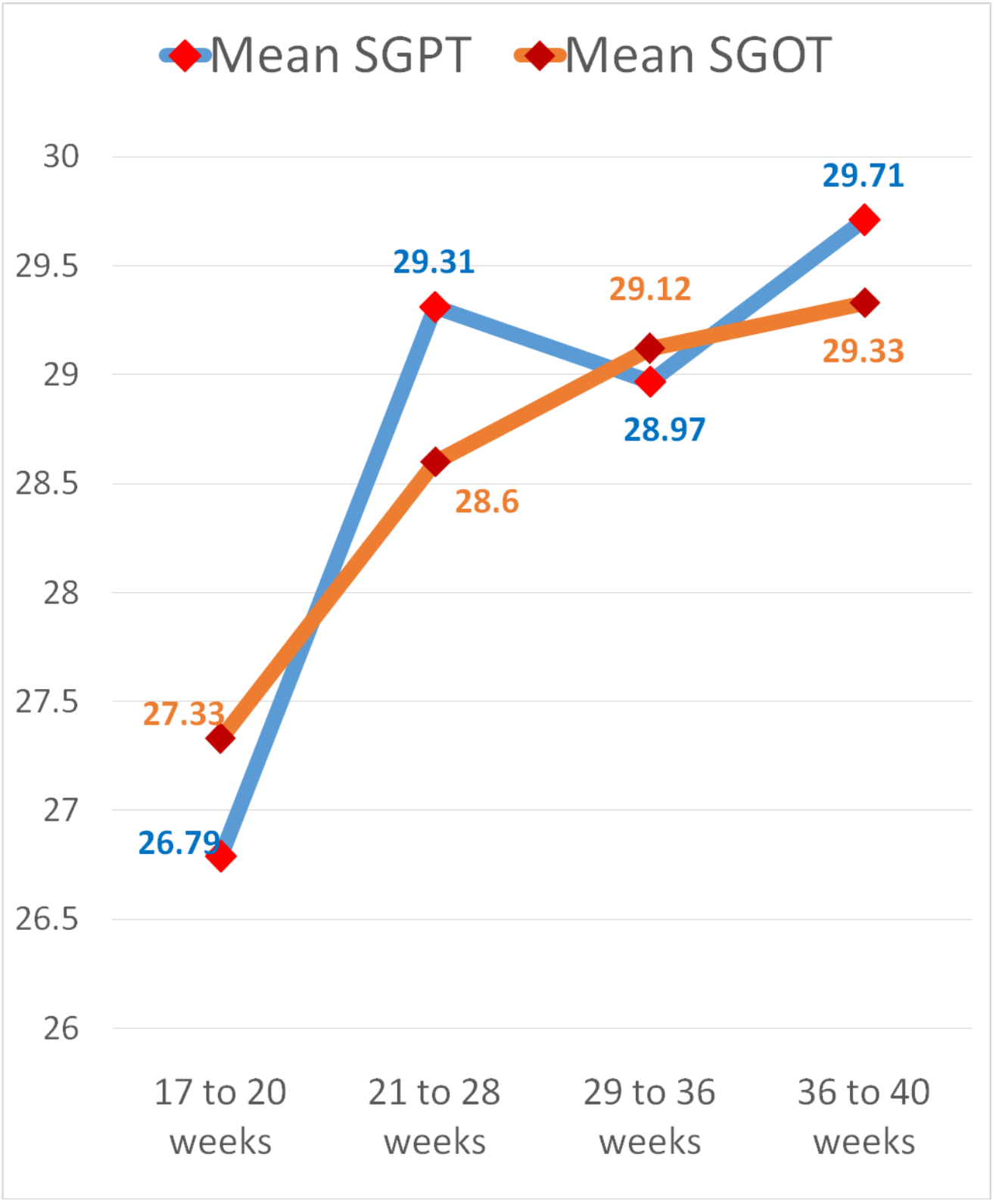


**Table 5 Distribution of the Mean maternal levels of Serum creatinine during various antenatal periods (n=90)**

| Serum creatinine levels | Mean | Standard Deviation | Minimum | Maximum |
|---|---|---|---|---|
| 17 to 20 weeks | 1.16 | 2.94 | 0.60 | 28.00 |
| 21 to 28 weeks | 0.87 | 0.06 | 0.80 | 1.10 |
| 29 to 36 weeks | 0.88 | 0.10 | 0.80 | 1.20 |
| 36 to 40 weeks | 0.92 | 0.12 | 0.80 | 1.30 |
| Difference between 1st and 4th reading | -0.24 | 2.91 | -26.80 | 0.40 |

**Figure 5:Line diagram showing mean serum creatinine levels during various antenatal periods (n=90)**

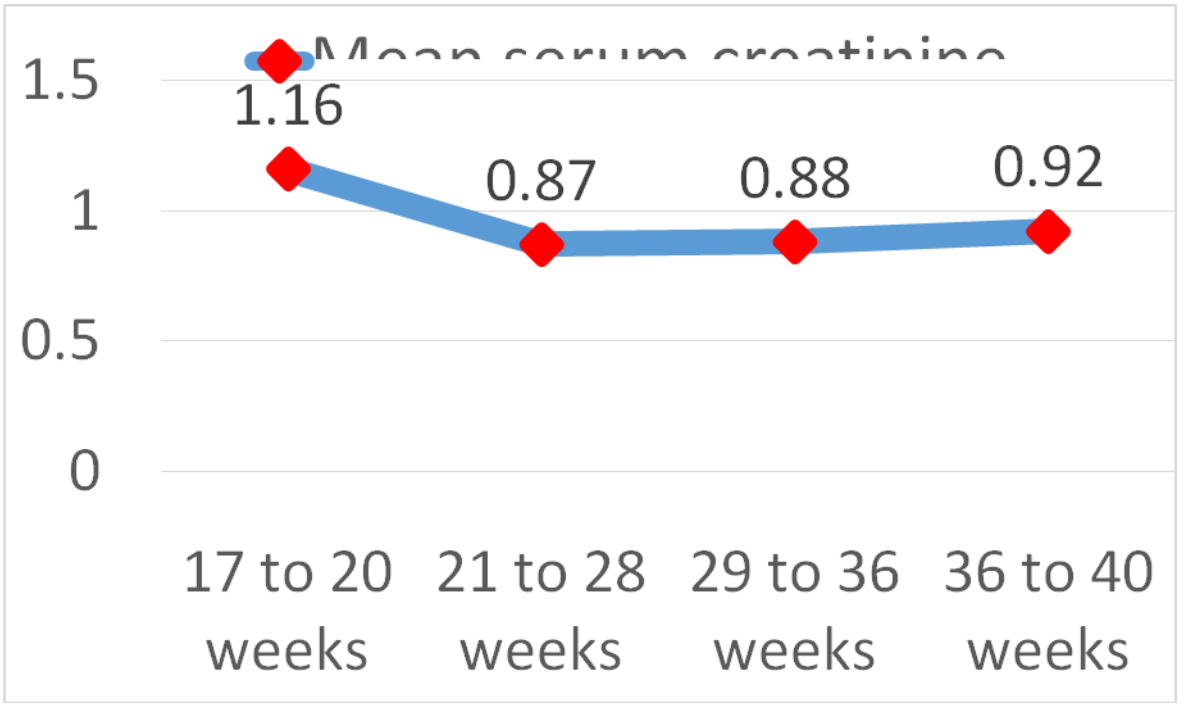


**Table 6 Distribution of the Mean maternal platelet count during various antenatal periods (n=90)**

| Platelet count | Mean | Standard Deviation | Minimum | Maximum |
|---|---|---|---|---|
| 17 to 20 weeks | 159381.9 | 6829.7 | 150085.0 | 176676.0 |
| 21 to 28 weeks | 153245.7 | 9302.0 | 98654.0 | 172538.0 |
| 29 to 36 weeks | 150519.1 | 18546.0 | 56000.0 | 170467.0 |
| 36 to 40 weeks | 149049.8 | 18397.3 | 67567.0 | 176069.0 |
| Difference between 1st and 4th reading | -10332.1 | 18755.0 | -92889.0 | 17974.0 |

**Figure 6:Line diagram showingmean platelet count during various antenatal periods (n=90)**

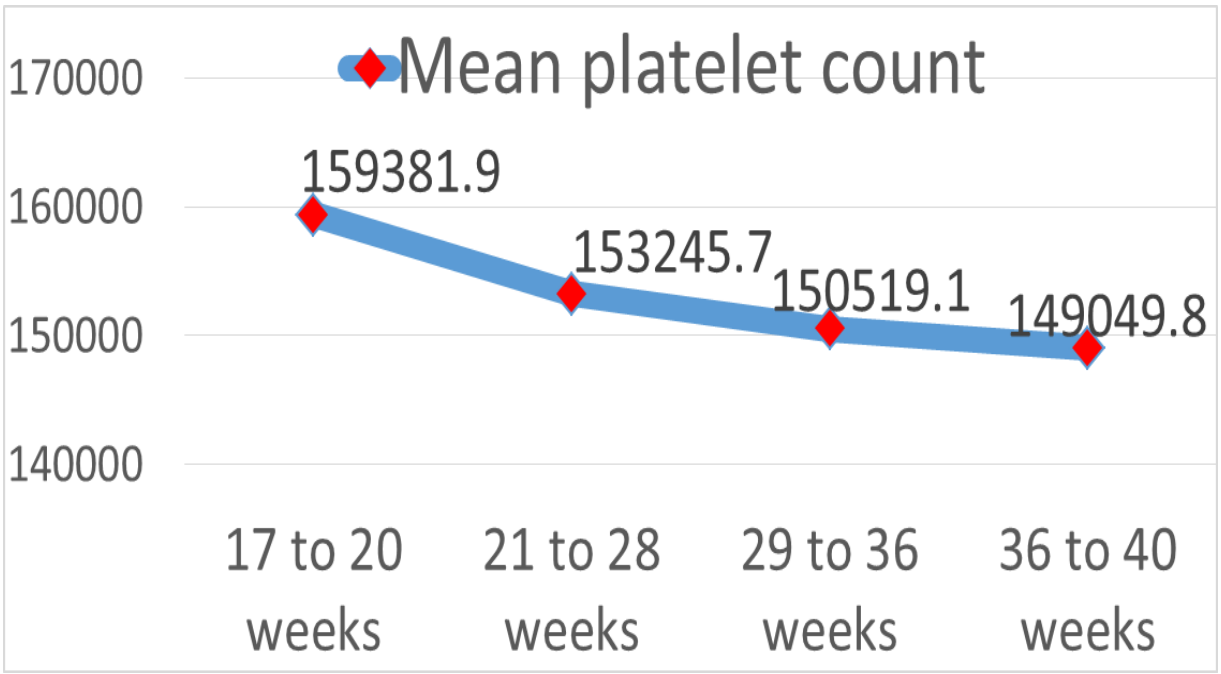
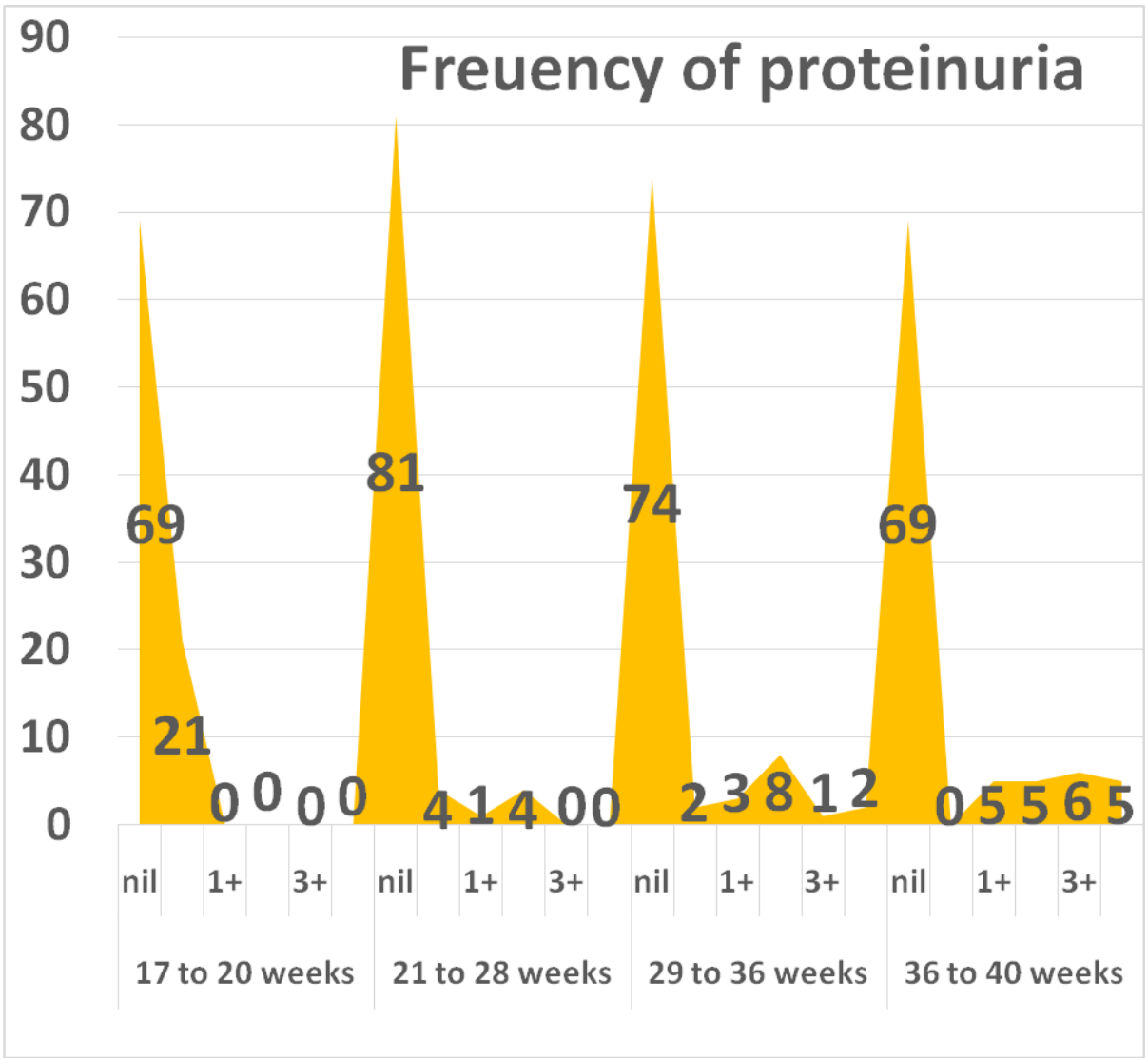


**Table 7 Distribution of the proteinuria during various antenatal periods (n=90)**

| Urinary protein | | Count | Column N % |
|---|---|---|---|
| 17 to 20 weeks | Nil | 69 | 76.7% |
| | Trace | 21 | 23.3% |
| 21 to 28 weeks | Nil | 81 | 90.0% |
| | Trace | 4 | 4.4% |
| | 1+ | 1 | 1.1% |
| | 2+ | 4 | 4.4% |
| 29 to 36 weeks | Nil | 74 | 82.2% |
| | Trace | 2 | 2.2% |
| | 1+ | 3 | 3.3% |
| | 2+ | 8 | 8.9% |
| | 3+ | 1 | 1.1% |
| | 4+ | 2 | 2.2% |
| 37 to 40 weeks | Nil | 69 | 76.7% |
| | 1+ | 5 | 5.6% |
| | 2+ | 5 | 5.6% |
| | 3+ | 6 | 6.7% |
| | 4+ | 5 | 5.6% |

**Figure 7 Showing proteinuria during various antenatal periods (n=90)**




There is increased proteinuria with corresponding gestational age in Preeclamptic and Eclamptic patients.

**Table 8 Distribution of the "soluble fms like tyrosine kinase 1" in the study population (n=90)**

| Descriptive statistic | "Soluble fms like tyrosine kinase 1" (sFlt in pg/ml) |
|---|---|
| Mean | 188.50 |
| Std. Deviation | 176.005 |
| Minimum | 76 |
| Maximum | 1409 |

| SFLT levels | Frequency | Percent |
|---|---|---|
| Normal (75 to 179 pg/ml ) | 71 | 78.9 |
| Elevated (> 179 pg/ml) | 19 | 21.1 |
| Total | 90 | 100.0 |

**Figure 8 Pie chart showing the levels of "soluble fms like tyrosine kinase 1" (n=90)**

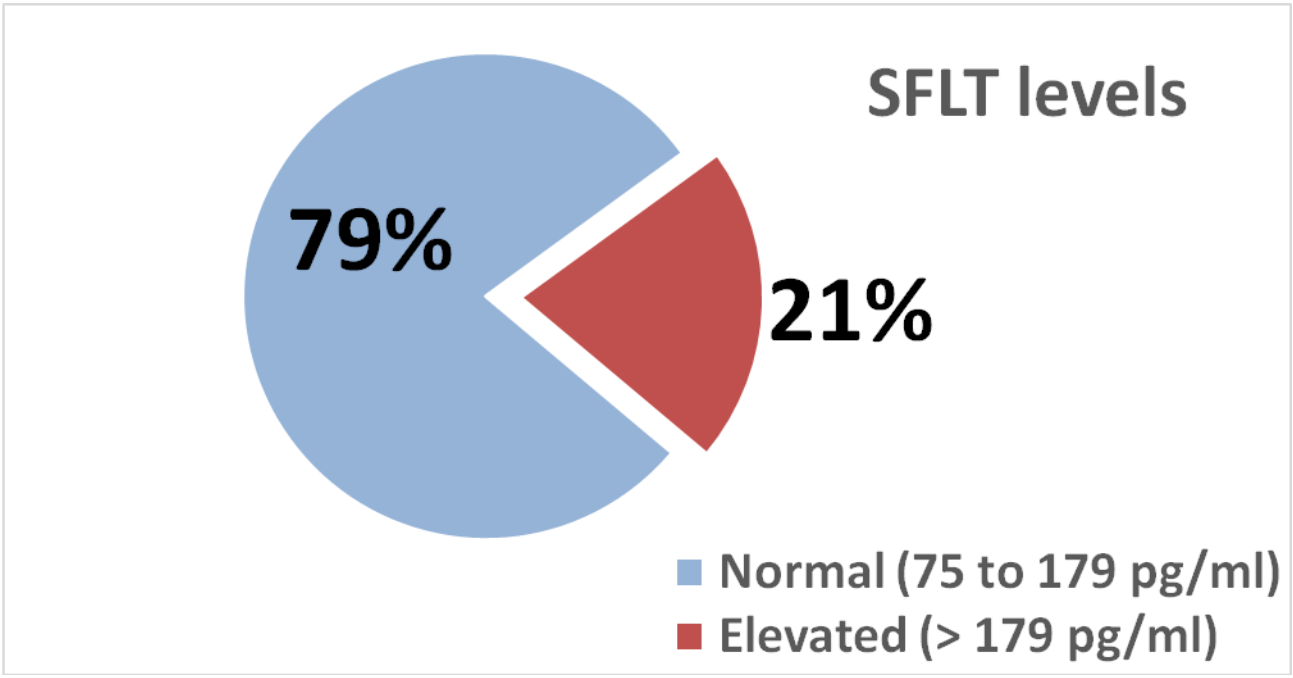
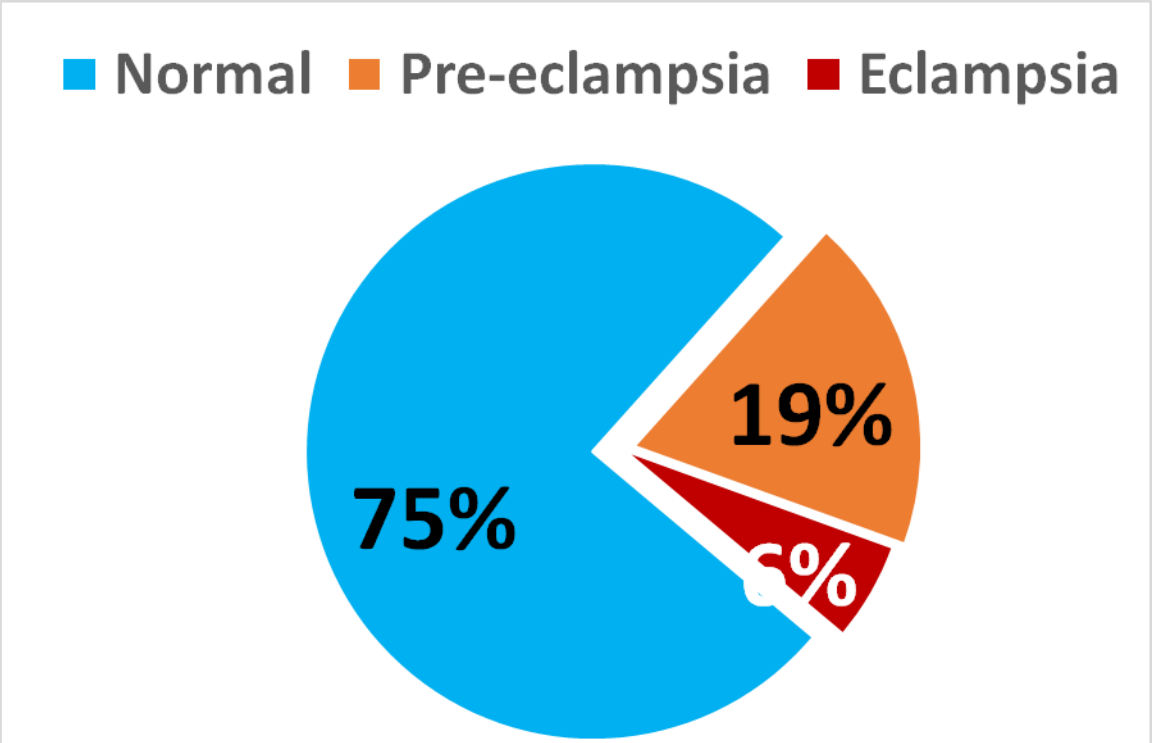


**Table 9 Distribution of the study population according to pre-eclampsia (n=90)**

| Medical condition | Frequency | Percent |
|---|---|---|
| Normal | 68 | 75.6 |
| Pre-eclampsia | 17 | 18.9 |
| Eclampsia | 5 | 5.6 |
| Total | 90 | 100.0 |

**Figure 9 Pie chart showingdistribution of the study population according to eclampsia (n=90)**




The "soluble fms like tyrosine kinase 1" levels were raised in 21% of the subjects with a mean level of 188.5 ± 176.0 pg/ml

**Table 10 Distribution of the study population according to pre-eclampsia and SFLT levels (n=90)**

| SFLT levels | Normal blood pressure N (%) | Pre-eclampsia & Eclampsia N (%) | Total N (%) |
|---|---|---|---|
| Normal (75 to 179 pg/ml) | 68 (100) | 3 (13.6) | 71(78.9) |
| Elevated (> 179 pg/ml) | 0 (0) | 19 (86.4) | 19 (21.1) |
| Total | 68 (100) | 22 (100) | 90 (100) |

**Figure 10 Bar chart showing distribution of the study population according to pre-eclampsia and SFLT levels (n=90)**

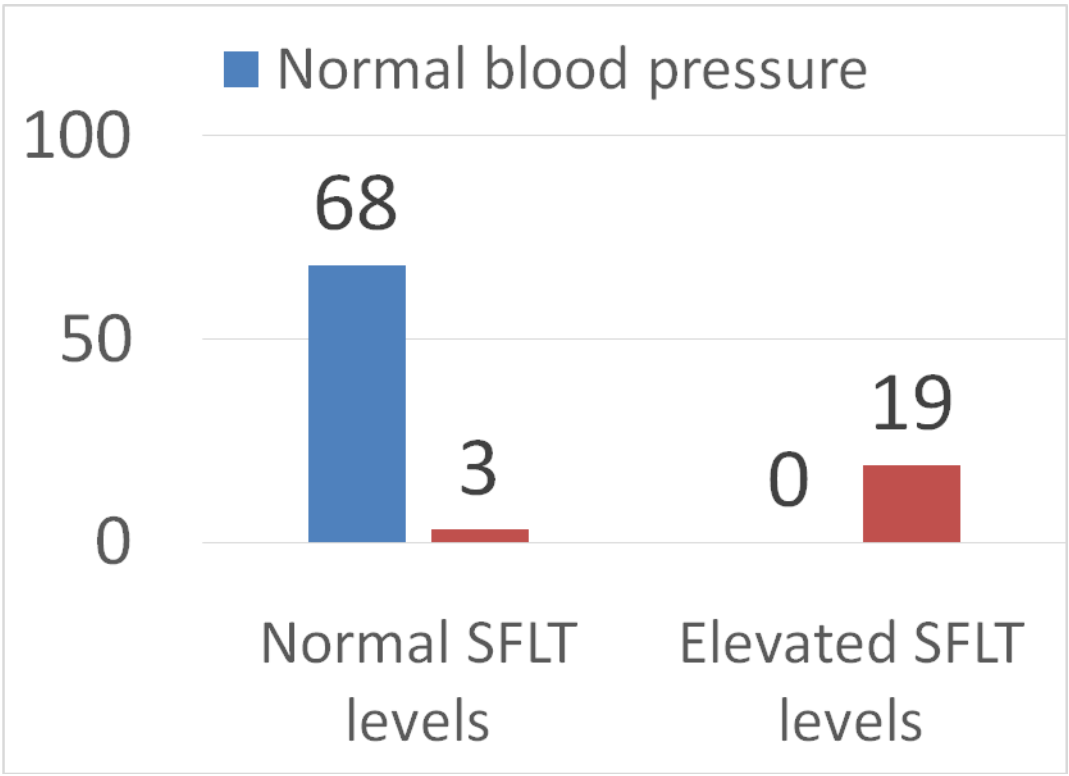


**Table 11 Distribution of the study population according to pre-eclampsia and SFLT levels (n=90)**

| SFLT levels | Normal blood pressure | Pre-eclampsia & Eclampsia | Total |
|---|---|---|---|
| Normal (75 to 179 pg/ml) N (%) | 68 (95.8) | 3 (4.2) | 71(100) |
| Elevated (> 179 pg/ml) N (%) | 0 (0) | 19 (100) | 19 (100) |
| Total N (%) | 68 (100) | 22 (100) | 90 (100) |

Relative risk = 100/4.22 = **23.69**

**Table 11 Distribution of SFLT levels of the study population according to pre-eclampsia (n=90)**

| Blood pressure | N | MeanSFLT levels | Std. Deviation | 95% Confidence Interval for Mean | |
|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound |
| Normal | 68 | 134.29 | 25.534 | 128.11 | 140.47 |
| Pre-eclampsia | 17 | 220.94 | 72.994 | 183.41 | 258.47 |
| Eclampsia | 5 | 815.40 | 333.678 | 401.08 | 1229.72 |
| Total | 90 | 188.50 | 176.005 | 151.64 | 225.36 |

ANOVA test was applied to test the difference in mean SFLT levels between the groups followed by Bonferroni post-Hoc test for inter-group comparisons.

**ANOVA test**

| | |
|---|---|
| p value | <0.001* |
| F statistic | 165.329 |
| Degree of freedom | 2 |

**Bonferroni Post-Hoc test**

| Comparison groups | Mean Difference | p value | 95% Confidence Interval For Mean Difference |
|---|---|---|---|
| Normal BP vs Pre-eclampsia | -86.647 | **0.001** | -140.43 to -32.87 |
| Normal BP vs Eclampsia | -681.106 | ***<0.001*** | -773.01 to -589.20 |
| Pre-eclampsia vs Eclampsia | -594.45 | ***<0.001*** | -695.36 to 493.56 |

**Table 12 Distribution of various parameters of the study population according to gestational age(n=90)**

| Difference between 17 weeks and 40 weeks | Mean difference (17 – 40 weeks) | Std. Deviation | 95% Confidence Interval of the Difference | | Paired "t" test p value |
|---|---|---|---|---|---|
| | | | Lower | Upper | |
| Maternal Weight | -12.311 | 2.048 | -12.74 | -11.88 | ***<0.001*** |
| Systolic pressure | -3.356 | 12.233 | -5.918 | -0.793 | ***0.011*** |
| Diastolic pressure | -2.278 | 9.701 | -4.310 | -0.246 | ***0.028*** |
| SGPT levels | -2.922 | 8.106 | -4.620 | -1.224 | ***0.001*** |
| SGOT levels | -2.000 | 7.733 | -3.620 | -0.380 | ***0.016*** |
| Serum creatinine levels | 0.2400 | 2.9073 | -0.3689 | 0.8489 | 0.436 |
| Platelet count | 10332.0 | 18754.9 | 6403.9 | 14260.2 | ***<0.001*** |

**Table 13: Pearson Correlation matrix between various parameters and SFLT levels (n=90)**

| | SFLT | Age | Systolic BP Rise | Diastolic BP Rise | SGPT Rise | SGOT Rise | Serum Creatinine fall | Platelet count fall |
|---|---|---|---|---|---|---|---|---|
| **SFLT** | 1 | | | | | | | |
| **Age** | -0.033 | 1 | | | | | | |
| **Systolic BP Rise** | 0.333* | 0.015 | 1 | | | | | |
| **Diastolic BP Rise** | 0.304* | -0.088 | 0.776* | 1 | | | | |
| **SGPT Rise** | 0.835* | -0.097 | 0.314* | 0.308* | 1 | | | |
| **SGOT Rise** | 0.849* | -0.01 | 0.334* | 0.307* | 0.836* | 1 | | |
| **Serum Creatinine fall** | -0.109 | 0.098 | -0.224* | -0.213* | -0.175 | 0.079 | 1 | |
| **Platelet count fall** | -0.695* | -0.027 | -0.094 | -0.015 | -0.761* | -0.771* | 0.01 | 1 |

*Correlation significant at 0.05 level

**Table 14: Pearson Correlation matrix between SFLT levels and various parameters among cases and controls (n=90)**

| Variables | Cases (n=20) | | Controls (n=45) | |
|---|---|---|---|---|
| | **Pearson Correlation** | **p value** | **Pearson Correlation** | **p value** |
| SFLT and Age | 0.123 | 0.586 | **-0.243** | **0.046** |
| SFLT and Rise in systolic BP | -0.334 | 0.129 | -0.225 | 0.065 |
| SFLT and Rise in diastolic BP | -0.326 | 0.059 | -0.099 | 0.420 |
| SFLT and Rise in ALT | **0.861** | **<0.001** | -0.007 | 0.954 |
| SFLT and Rise in AST | **0.841** | **<0.001** | 0.210 | 0.086 |
| SFLT and Fall in serum creatinine | 0.006 | 0.978 | 0.046 | 0.712 |
| SFLT and Fall in platelet count | **-0.748** | **<0.001** | -0.107 | 0.385 |

**Table 15 Logistic regression of various factors with Occurrence of pre-eclampsia or eclampsia as dependent variable (n=90)**

| INDEPENDENT VARIABLE (N) | Odds Ratio (95% CI) | p value | Adjusted Odds Ratio (95% CI) | p value |
|---|---|---|---|---|
| SFLT levels (pg/ml) | 1.049 (1.021 to 1.078) | **0.001** | 1.048 (1.012 to 1.085) | **0.008** |
| Age (in years) | 0.898 (0.726 to 1.112) | 0.323 | 0.810 (0.494 to 1.330) | 0.406 |
| Mean Weight gain during pregnancy (in kg) | 1.563 (1.150 to 2.126) | **0.004** | 2.233 (1.088 to 4.584) | **0.029** |
| Mean Rise in SGPT during pregnancy | 1.349 (1.134 to 1.605) | **0.001** | 0.887 (0.630 to 1.247) | 0.489 |
| Mean rise in SGOT during pregnancy | 1.334 (1.133 to 1.571) | **0.001** | 1.189 (0.816 to 1.733) | 0.367 |
| Fall in Platelet count during pregnancy | 0.999974 (0.999950 to 0.999999) | 0**.040** | 1.000172 (1.000042 to 1.000303) | **0.010** |

Nagelkerke's Pseudo R square for multivariate model: 79.8%

# DISCUSSION

## DISCUSSION

"Hypertensive disorders of pregnancy" are considered to be one of the major health problem worldwide, causing an increased perinatal and maternal morbidity and mortality[65]. Numerous complex mechanisms play an important role in trophoblastic-endothelial dysfunction which includes altered nitric oxide production, lipid and protein oxidation, anti angiogenic factors and adhesion molecules. All these factors can be suggested as the etiopathogenesis of Preeclampsia.

Increase in placental derived "soluble fms-like tyrosine kinase 1' (sFlt-1) or the "soluble vascular endothelial growth factor (VEGF) receptor 1" are responsible for the signs and symptoms of Preeclampsia, and increased levels of these circulating markers are associated with Preeclampsia. The incidence of gestational hypertension in developing countries and the highly industrialized world is similar but incidence of Preeclampsia & Eclampsia are higher in developing countries due to poor antenatal care and lack of education. The following factors may help to improve maternal and perinatal outcomes

1. Early detection of high-risk individuals by trained personnel
2. Timely referral to tertiary centres
3. Early and timely treatment of Preeclampsia cases
4. Correct training of the mothers about fertility age and the importance of care during pregnancy

In our study , we focused only on primigravida, because nulliparity itself is a main risk factor for the development of Preeclampsia.

If we look into distribution of study population according to Preeclampsia and SFLT levels through Pearson's Chi-square with continuity correction( 69.348, p value: <0.001), there is a statistically significant association between elevated SFLT levels and occurrence of Preeclampsia with 86.4% of Pre-eclampsia & Eclampsia patients showing elevated SFLT levels.

In our study population , Preeclampsia was present in 19% of subjects and Eclampsia in 5.6% of the subjects.

There is a statistically significant positive linear correlation between SFLT levels and levels of Systolic BP, Diastolic BP, ALT and AST i.e., an increase in SFLT level is associated with corresponding increase in these parameters .

There is a statistically significant negative linear correlation between SFLT levels and Platelet count. i.e, an increase in SFLT levels with corresponding decrease in Platelet count .

Table 1 depicts age distribution of study population showing mean age of the population as 25 years with 57.8% of population belonging to 21-25 years age group.

Table 2 shows distribution of mean maternal weight in kg during various antenatal period . The mean weight gain during pregnancy is 12.3 kg in our study

Table 3 shows distribution of the Mean maternal blood pressure during various antenatal periods revealing 3 mmHg raise in mean systolic pressure and 2 mmHg in mean diastolic pressure during pregnancy.

Various studies show that all preeclamptic women had significantly higher levels of sFlt-1 at gestational ages where their blood pressure was within normal limits.

Table 4 shows distribution of the mean maternal levels of transaminases during various antenatal periods. The mean ALT levels are raised by approximately 3 units and AST levels by 2 units during pregnancy.

During the course of pregnancy from 17 weeks to term, many parameters like maternal Weight, systolic pressure, diastolic pressure, ALT and AST levels increased with Eclampsia corresponding to gestational age and this elevation during pregnancy is statistically significant.

Table 5 shows distribution of the mean maternal levels of Serum creatinine during various antenatal periods .It shows a decline in mean creatinine levels initially during pregnancy and then raised marginally towards term in eclampsia. The difference of mean serum creatinine levels between 17 weeks and term was not statistically significant.

Table 6 shows distribution of the Mean maternal platelet count during various antenatal periods. The mean maternal platelet count showed a gradual decline with increase in gestational age in eclampsia patients which is statistically significant.

Table 7 shows distribution of the proteinuria during various antenatal periods. There is increased proteinuria with corresponding gestational age in preeclamptic and eclamptic patients.

Table 8 shows distribution of the "soluble fms like tyrosine kinase 1" in the study population .The "soluble fms like tyrosine kinase 1" levels were elevated in 21% of the subjects with a mean level of 188.5 ± 176.0 pg/ml.

Table 9 shows the presence of Pre-eclampsia in 19% of subjects and eclampsia in 5.6% of the subjects in our study.

Table 10 Distribution of the study population according to pre-eclampsia and SFLT levels shows Relative risk = 100/4.22 = 23.69

Subjects with elevated SFLT levels have 23 times higher risk of developing Pre-eclampsia & Eclampsia in comparison to Subjects with normal SFLT levels.

During univariate analysis, an increase in variables like SFLT levels, mean weight gain (in kg), mean rise in ALT & AST levels during pregnancy were

significantly associated with raised risk of developing pre-eclampsia and eclampsia.

During multivariate analysis, there was statistically significant increased risk of pre-eclampsia with rise in SFLT levels, rise in mean weight gain and fall in platelet count. But the mean rise in ALT & AST levels during pregnancy was not significant.

Nagelkerke's Pseudo R Square shows that in the multivariate model, 79.8% of variability in the occurrence of pre-eclampsia can be explained by the variables in the model.

ANOVA test was applied to test the difference in mean SFLT levels between the groups followed by Bonferroni post-Hoc test for inter-group comparisons. ANOVA test showed that there is a statistically significant difference in the mean SFLT levels between the 3 groups according to maternal blood pressure.

Bonferroni test showed that the difference in mean SFLT levels between all the groups was statistically significant (p value <0.05) and that mean SFLT levels increases with increase in blood pressure and occurrence of eclampsia.

Moreover, mean SFLT levels between the groups pre-eclampsia vs eclampsia was also statistically significant (p value <0.05) as SFLT levels were raised remarkably in cases of eclampsia.

# CONCLUSION

## CONCLUSION

From this study, It is found that the primigravida with increased serum sFlt levels had developed Preeclampsia in the later pregnancy and few of them developed Eclampsia . So , serum sFlt levels can be used to predict the occurrence of both Preeclampsia and progression To Eclampsia.

## FUTURE SCOPE OF THIS STUDY:

Till date the only means of treating Preeclampsia is delivery. Many nonspecific interventions like using antihypertensive agents have been tried, but with limited success. There is less evidence to support whether these interventions can resolve the clinical manifestations or prevent them from developing. Also there is no data whether these interventions can prolong a preterm pregnancy complicated by Preeclampsia . The factors responsible for the pathogenesis of Preeclampsia is not clear , making new treatment strategies impossible.

In view of early diagnosis of this condition , many new soluble angiogenic are being discovered which can be used either in isolation or 2 or 3 markers can be combined and used in predicting or early diagnosis of Preeclampsia. Hence predicting Preeclampsia or early diagnosis of Preeclampsia by using these markers can help in development of new treatment modalities

There is only limited success by removing toxic circulating factors responsible for Preeclampsia by either hemodialysis or plasmapheresis. But none of them have prolonged a preterm pregnancy complicated by Preeclampsia. Circulating sFlt-1 accounts for less than 20% of the total body sFlt-1 burden. Creating a selective adsorption column with a concentration gradient may increase the removal of these toxic substances..

## LIMITATIONS OF THE STUDY

In view of diagnosing the condition , the development of soluble angiogenic markers discussed above help in the screening and early diagnosis of Preeclampsia. Most of the studies which support the use of these markers in screening and in early diagnosis are retrospective. There is definitely a need for more longitudinal prospective studies to substantiate their role as a reliable marker in identifying women at risk and in early diagnosis in those who develop Preeclampsia [52]

# ANNEXURE

# BIBLIOGRAPHY

## BIBLIOGRAPHY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# MASTERCHART

| SI NO | AGE | WEEKS | | | WEIGHT | | | BLOODPRESSURE | | | | | | ALT | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 17-20 | 21-28 | 29-36 | 36-40 | POSTNATAL | 1 | 2 | 3 | 4 |
| 1 | 22 | 18 | 45 | 47 | 49 | 55 | 122/86 | 130/88 | 124/88 | 148/106 | 130/86 | 33 | 36 | 28 | 37 |
| 2 | 23 | 19 | 53 | 53 | 60 | 65 | 120/89 | 106/70 | 116/80 | 146/100 | 120/89 | 30 | 27 | 23 | 24 |
| 3 | 27 | 18 | 55 | 56 | 62 | 68 | 116/80 | 104/76 | 112/80 | 112/80 | 126/80 | 23 | 28 | 24 | 25 |
| 4 | 25 | 17 | 60 | 61 | 66 | 73 | 112/80 | 90/60 | 110/76 | 110/76 | 112/86 | 28 | 29 | 25 | 26 |
| 5 | 25 | 18 | 65 | 66 | 73 | 79 | 110/76 | 92/66 | 106/86 | 150/110 | 110/76 | 29 | 27 | 27 | 24 |
| 6 | 24 | 18 | 53 | 57 | 60 | 65 | 130/86 | 122/80 | 116/86 | 140/90 | 120/86 | 32 | 33 | 37 | 36 |
| 7 | 28 | 19 | 55 | 56 | 62 | 68 | 116/80 | 104/76 | 112/80 | 112/80 | 106/80 | 25 | 23 | 28 | 24 |
| 8 | 24 | 18 | 53 | 57 | 60 | 65 | 130/86 | 122/80 | 116/86 | 140/90 | 136/86 | 32 | 33 | 37 | 36 |
| 9 | 21 | 18 | 65 | 66 | 70 | 75 | 120/76 | 116/70 | 106/80 | 116/80 | 120/60 | 26 | 24 | 28 | 24 |
| 10 | 26 | 18 | 60 | 60 | 66 | 73 | 106/80 | 100/60 | 122/80 | 128/80 | 116/80 | 24 | 26 | 24 | 26 |
| 11 | 23 | 19 | 66 | 66 | 73 | 79 | 116/80 | 104/76 | 112/80 | 112/80 | 106/80 | 25 | 28 | 22 | 28 |
| 12 | 29 | 17 | 65 | 65 | 70 | 75 | 124/86 | 146/100 | 160/106 | 140/92 | 124/90 | 35 | 45 | 68 | 56 |
| 13 | 30 | 18 | 65 | 65 | 69 | 75 | 120/89 | 106/70 | 116/80 | 116/80 | 120/89 | 26 | 24 | 28 | 24 |
| 14 | 28 | 19 | 60 | 61 | 66 | 73 | 106/80 | 104/76 | 112/80 | 112/80 | 106/80 | 24 | 26 | 24 | 26 |
| 15 | 24 | 18 | 65 | 66 | 70 | 74 | 116/80 | 104/86 | 112/80 | 102/80 | 116/80 | 25 | 28 | 22 | 28 |
| 16 | 22 | 19 | 58 | 64 | 68 | 72 | 126/88 | 124/86 | 112/88 | 140/90 | 126/88 | 28 | 37 | 32 | 33 |
| 17 | 23 | 18 | 55 | 56 | 62 | 68 | 120/89 | 106/70 | 116/80 | 116/80 | 106/80 | 30 | 27 | 23 | 24 |
| 18 | 24 | 17 | 50 | 51 | 56 | 63 | 116/80 | 104/76 | 90/80 | 112/80 | 106/70 | 23 | 28 | 24 | 25 |
| 19 | 25 | 18 | 60 | 61 | 66 | 73 | 106/80 | 104/78 | 112/80 | 104/80 | 106/80 | 28 | 29 | 25 | 26 |
| 20 | 22 | 19 | 56 | 58 | 62 | 68 | 120/84 | 114/76 | 124/96 | 144/96 | 120/84 | 33 | 37 | 36 | 33 |
| 21 | 21 | 19 | 60 | 61 | 66 | 73 | 120/89 | 106/70 | 116/80 | 116/80 | 116/80 | 24 | 26 | 23 | 28 |
| 22 | 23 | 20 | 65 | 66 | 70 | 74 | 116/80 | 104/76 | 112/80 | 112/80 | 116/80 | 22 | 28 | 28 | 29 |
| 23 | 28 | 18 | 64 | 66 | 73 | 77 | 126/86 | 116/86 | 124/90 | 152/100 | 126/86 | 28 | 37 | 36 | 28 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 24 | 30 | 19 | 60 | 61 | 66 | 73 | 106/80 | 100/60 | 122/80 | 128/80 | 120/89 | 25 | 29 | 25 | 26 |
| 25 | 26 | 18 | 60 | 61 | 66 | 73 | 116/80 | 104/76 | 112/80 | 112/80 | 116/80 | 23 | 28 | 24 | 25 |
| 26 | 27 | 20 | 64 | 65 | 70 | 74 | 124/86 | 144/102 | 156/110 | 132/86 | 124/86 | 38 | 42 | 61 | 66 |
| 27 | 26 | 18 | 58 | 57 | 62 | 68 | 120/89 | 106/70 | 116/80 | 116/80 | 120/89 | 23 | 28 | 24 | 25 |
| 28 | 23 | 19 | 60 | 61 | 66 | 73 | 116/80 | 104/76 | 112/80 | 112/80 | 120/89 | 28 | 29 | 25 | 26 |
| 29 | 28 | 18 | 60 | 61 | 66 | 73 | 106/80 | 100/60 | 122/80 | 128/80 | 106/80 | 29 | 27 | 27 | 24 |
| 30 | 29 | 19 | 52 | 54 | 61 | 70 | 126/86 | 134/88 | 134/94 | 144/108 | 126/86 | 33 | 36 | 32 | 37 |
| 31 | 25 | 17 | 65 | 65 | 69 | 75 | 120/89 | 90/70 | 106/80 | 116/80 | 106/70 | 30 | 27 | 23 | 24 |
| 32 | 26 | 18 | 60 | 61 | 66 | 73 | 116/80 | 104/76 | 112/80 | 112/80 | 116/80 | 23 | 28 | 24 | 25 |
| 33 | 23 | 18 | 65 | 66 | 70 | 74 | 120/89 | 106/70 | 116/80 | 116/80 | 120/89 | 28 | 29 | 25 | 26 |
| 34 | 25 | 19 | 55 | 60 | 64 | 70 | 126/80 | 116/86 | 144/90 | 156/110 | 126/80 | 33 | 36 | 38 | 40 |
| 35 | 26 | 18 | 60 | 61 | 66 | 73 | 112/80 | 90/60 | 110/76 | 110/76 | 106/70 | 25 | 29 | 25 | 26 |
| 36 | 26 | 18 | 60 | 61 | 66 | 73 | 116/80 | 104/76 | 112/80 | 112/80 | 118/80 | 30 | 27 | 23 | 24 |
| 37 | 24 | 19 | 64 | 64 | 69 | 75 | 132/86 | 156/100 | 146/110 | 140/94 | 132/86 | 33 | 44 | 65 | 70 |
| 38 | 25 | 18 | 55 | 56 | 62 | 68 | 116/80 | 104/76 | 112/80 | 112/80 | 120/89 | 24 | 26 | 23 | 28 |
| 39 | 25 | 19 | 60 | 61 | 66 | 73 | 112/80 | 90/60 | 110/76 | 110/76 | 106/70 | 22 | 28 | 28 | 29 |
| 40 | 28 | 18 | 60 | 61 | 66 | 73 | 112/80 | 90/60 | 110/76 | 116/76 | 122/80 | 24 | 25 | 24 | 26 |
| 41 | 27 | 19 | 55 | 56 | 62 | 68 | 112/80 | 90/60 | 110/76 | 110/76 | 112/80 | 24 | 28 | 24 | 25 |
| 42 | 25 | 18 | 66 | 66 | 71 | 77 | 128/88 | 144/100 | 140/110 | 146/100 | 128/88 | 34 | 43 | 66 | 69 |
| 43 | 26 | 18 | 65 | 65 | 69 | 75 | 112/80 | 90/60 | 110/76 | 110/76 | 112/80 | 30 | 27 | 23 | 24 |
| 44 | 27 | 19 | 60 | 61 | 66 | 73 | 116/80 | 104/76 | 112/80 | 112/80 | 106/70 | 23 | 28 | 24 | 25 |
| 45 | 23 | 19 | 65 | 66 | 70 | 74 | 112/80 | 90/60 | 110/76 | 110/76 | 116/80 | 28 | 29 | 25 | 26 |
| 46 | 22 | 18 | 55 | 63 | 68 | 72 | 130/88 | 124/88 | 140/96 | 154/110 | 130/88 | 32 | 36 | 39 | 40 |
| 47 | 21 | 19 | 55 | 56 | 62 | 68 | 126/80 | 104/76 | 112/80 | 112/80 | 126/80 | 24 | 26 | 23 | 28 |
| 48 | 28 | 18 | 60 | 61 | 66 | 73 | 112/80 | 90/60 | 110/76 | 108/76 | 116/80 | 22 | 28 | 28 | 29 |
| 49 | 28 | 19 | 60 | 61 | 66 | 73 | 106/80 | 104/76 | 112/80 | 112/80 | 106/80 | 24 | 26 | 24 | 26 |
| 50 | 27 | 18 | 55 | 56 | 62 | 68 | 1180 | 90/60 | 110/76 | 110/76 | 112/80 | 24 | 28 | 24 | 25 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 51 | 26 | 18 | 58 | 65 | 69 | 74 | 130/78 | 126/88 | 146/96 | 144/110 | 130/78 | 32 | 33 | 38 | 39 |
| 52 | 26 | 19 | 60 | 61 | 66 | 73 | 120/89 | 106/70 | 106/80 | 116/80 | 106/70 | 26 | 24 | 28 | 24 |
| 53 | 23 | 18 | 65 | 66 | 73 | 79 | 116/80 | 104/76 | 112/80 | 122/80 | 116/80 | 24 | 26 | 24 | 26 |
| 54 | 24 | 18 | 55 | 56 | 62 | 68 | 112/80 | 90/60 | 110/76 | 110/76 | 112/80 | 25 | 28 | 22 | 28 |
| 55 | 23 | 18 | 57 | 60 | 64 | 70 | 120/88 | 124/88 | 146/96 | 150/110 | 120/88 | 30 | 34 | 39 | 39 |
| 56 | 22 | 19 | 53 | 53 | 60 | 65 | 126/80 | 104/76 | 96/80 | 112/80 | 126/76 | 25 | 24 | 26 | 24 |
| 57 | 23 | 18 | 55 | 56 | 62 | 68 | 112/80 | 90/60 | 110/76 | 108/76 | 116/80 | 22 | 28 | 25 | 29 |
| 58 | 23 | 20 | 65 | 66 | 70 | 74 | 116/80 | 104/76 | 112/80 | 112/80 | 116/80 | 22 | 28 | 28 | 29 |
| 59 | 21 | 18 | 58 | 59 | 65 | 71 | 122/86 | 140/100 | 148/110 | 146/96 | 122/86 | 32 | 47 | 67 | 69 |
| 60 | 26 | 19 | 60 | 61 | 66 | 73 | 116/80 | 104/76 | 112/80 | 102/80 | 126/80 | 24 | 28 | 24 | 25 |
| 61 | 27 | 18 | 54 | 56 | 60 | 72 | 130/88 | 124/88 | 140/96 | 154/110 | 130/88 | 32 | 36 | 39 | 40 |
| 62 | 28 | 19 | 60 | 62 | 66 | 72 | 106/80 | 104/76 | 112/70 | 112/80 | 104/80 | 26 | 24 | 28 | 24 |
| 63 | 21 | 18 | 65 | 65 | 69 | 75 | 102/80 | 96/60 | 110/86 | 110/76 | 102/80 | 24 | 26 | 24 | 26 |
| 64 | 22 | 19 | 60 | 61 | 66 | 73 | 116/80 | 104/76 | 102/70 | 112/80 | 106/80 | 25 | 28 | 22 | 28 |
| 65 | 23 | 18 | 65 | 66 | 70 | 74 | 112/80 | 90/60 | 110/76 | 110/76 | 102/80 | 25 | 24 | 26 | 24 |
| 66 | 25 | 19 | 58 | 65 | 69 | 74 | 130/88 | 126/88 | 146/96 | 144/110 | 130/88 | 32 | 33 | 38 | 39 |
| 67 | 26 | 18 | 60 | 61 | 66 | 73 | 116/80 | 104/76 | 112/80 | 112/80 | 118/80 | 30 | 27 | 23 | 24 |
| 68 | 21 | 17 | 65 | 65 | 73 | 79 | 102/80 | 106/60 | 110/76 | 110/86 | 112/80 | 23 | 28 | 24 | 25 |
| 69 | 26 | 17 | 60 | 61 | 66 | 73 | 116/80 | 104/76 | 112/80 | 112/80 | 116/80 | 28 | 29 | 25 | 26 |
| 70 | 23 | 18 | 57 | 60 | 68 | 72 | 130/88 | 124/88 | 140/96 | 154/110 | 130/88 | 32 | 36 | 39 | 40 |
| 71 | 25 | 18 | 60 | 61 | 65 | 73 | 124/80 | 100/76 | 112/80 | 112/80 | 114/80 | 24 | 28 | 24 | 25 |
| 72 | 25 | 17 | 65 | 64 | 69 | 73 | 122/80 | 108/60 | 100/76 | 106/76 | 126/80 | 24 | 26 | 24 | 24 |
| 73 | 27 | 19 | 60 | 61 | 66 | 73 | 116/80 | 104/76 | 112/80 | 112/80 | 106/70 | 23 | 28 | 24 | 25 |
| 74 | 26 | 18 | 60 | 61 | 66 | 73 | 116/80 | 104/76 | 112/80 | 112/80 | 118/80 | 30 | 27 | 23 | 24 |
| 75 | 21 | 19 | 67 | 66 | 68 | 75 | 116/80 | 104/76 | 122/80 | 112/80 | 106/80 | 24 | 30 | 27 | 23 |
| 76 | 26 | 19 | 60 | 61 | 66 | 73 | 120/89 | 100/70 | 116/80 | 116/80 | 106/89 | 25 | 23 | 28 | 24 |
| 77 | 25 | 18 | 65 | 66 | 70 | 74 | 116/80 | 104/76 | 112/80 | 110/80 | 126/80 | 24 | 26 | 24 | 24 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 78 | 24 | 17 | 50 | 51 | 56 | 63 | 116/80 | 104/76 | 90/80 | 112/80 | 106/70 | 23 | 28 | 24 | 25 |
| 79 | 28 | 18 | 65 | 65 | 69 | 75 | 116/80 | 100/76 | 112/80 | 112/80 | 126/80 | 25 | 24 | 26 | 24 |
| 80 | 21 | 19 | 59 | 60 | 64 | 68 | 116/80 | 104/76 | 100/80 | 112/80 | 118/80 | 24 | 28 | 24 | 25 |
| 81 | 26 | 18 | 65 | 66 | 70 | 74 | 112/80 | 90/60 | 110/76 | 110/76 | 122/80 | 24 | 30 | 27 | 23 |
| 82 | 25 | 17 | 65 | 65 | 69 | 75 | 120/89 | 90/70 | 106/80 | 116/80 | 106/70 | 30 | 27 | 23 | 24 |
| 83 | 24 | 19 | 60 | 60 | 65 | 73 | 120/89 | 106/70 | 116/80 | 116/80 | 120/76 | 26 | 24 | 28 | 24 |
| 84 | 25 | 18 | 55 | 56 | 62 | 68 | 126/80 | 104/76 | 112/80 | 122/80 | 116/80 | 24 | 26 | 24 | 26 |
| 85 | 26 | 19 | 60 | 61 | 66 | 73 | 116/80 | 104/86 | 112/80 | 116/80 | 106/80 | 25 | 28 | 22 | 28 |
| 86 | 23 | 18 | 55 | 56 | 62 | 68 | 112/80 | 90/60 | 110/76 | 108/76 | 116/80 | 22 | 28 | 25 | 29 |
| 87 | 28 | 18 | 60 | 61 | 66 | 73 | 112/80 | 90/60 | 110/76 | 116/76 | 122/80 | 24 | 25 | 24 | 26 |
| 88 | 25 | 18 | 65 | 66 | 70 | 75 | 116/80 | 104/76 | 90/80 | 112/80 | 118/80 | 26 | 24 | 26 | 24 |
| 89 | 23 | 18 | 57 | 60 | 68 | 72 | 130/88 | 124/88 | 140/96 | 154/110 | 130/88 | 32 | 36 | 39 | 40 |
| 90 | 28 | 19 | 60 | 61 | 66 | 73 | 116/80 | 104/76 | 112/80 | 112/80 | 126/80 | 24 | 28 | 24 | 25 |

# MASTER CHART

| | | AST | | | SERUM CREATININE | | | | | PLATELET COUNT | | | | URINE PROTEIN | | SFLT pg/ml |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 |
| 32 | 33 | 37 | 34 | 0.9 | 1.1 | 0.8 | 0.9 | 1,50,085 | 1,50,069 | 1,55,343 | 1,56,324 | nil | nil | nil | 1+ | 203 |
| 26 | 24 | 28 | 24 | 0.6 | 0.8 | 0.8 | 1 | 1,55,678 | 1,56,789 | 1,55,769 | 1,55,655 | nil | trace | trace | 2+ | 78 |
| 24 | 26 | 24 | 26 | 0.8 | 0.9 | 0.9 | 0.8 | 1,54,356 | 1,53,678 | 1,54,324 | 1,52,455 | nil | nil | nil | nil | 88 |
| 25 | 28 | 22 | 28 | 0.9 | 0.9 | 0.8 | 0.9 | 1,67,895 | 1,51,234 | 1,54,673 | 1,51,234 | trace | nil | nil | nil | 76 |
| 27 | 23 | 26 | 26 | 0.9 | 0.9 | 0.8 | 0.9 | 1,56,784 | 1,54,678 | 1,55,678 | 1,56,456 | nil | nil | 1+ | 2+ | 97 |
| 33 | 36 | 28 | 37 | 0.9 | 0.8 | 1 | 0.8 | 1,50,567 | 1,60,198 | 1,60,678 | 1,50,564 | nil | nil | nil | 1+ | 187 |
| 27 | 27 | 24 | 27 | 0.8 | 0.9 | 0.9 | 0.8 | 1,67,895 | 1,51,234 | 1,54,673 | 1,51,234 | nil | nil | nil | nil | 102 |
| 33 | 36 | 28 | 37 | 0.9 | 0.8 | 1 | 0.8 | 1,50,567 | 1,50,198 | 1,50,678 | 1,50,564 | nil | nil | nil | 1+ | 201 |
| 30 | 27 | 23 | 24 | 0.8 | 0.9 | 0.9 | 0.8 | 1,55,678 | 1,56,789 | 1,55,769 | 1,55,655 | nil | trace | nil | nil | 112 |
| 23 | 28 | 24 | 25 | 0.9 | 0.9 | 0.8 | 0.9 | 1,54,356 | 1,53,678 | 1,54,324 | 1,52,455 | nil | nil | nil | nil | 79 |
| 28 | 29 | 25 | 26 | 0.9 | 0.9 | 0.8 | 0.9 | 1,67,895 | 1,51,234 | 1,54,673 | 1,51,234 | nil | nil | nil | nil | 156 |
| 38 | 46 | 69 | 61 | 0.9 | 1 | 1.2 | 1.3 | 1,60,623 | 1,60,000 | 96,000 | 80000E | NIL | 2+ | 2+ | 4+ | 639 |
| 23 | 28 | 24 | 25 | 0.6 | 0.8 | 0.8 | 1 | 1,55,678 | 1,56,789 | 1,55,769 | 1,55,655 | nil | nil | nil | nil | 145 |
| 28 | 29 | 25 | 26 | 0.8 | 0.9 | 0.9 | 0.8 | 1,54,356 | 1,53,678 | 1,54,324 | 1,52,455 | trace | nil | nil | nil | 123 |
| 29 | 27 | 27 | 24 | 0.9 | 0.9 | 0.8 | 0.9 | 1,67,895 | 1,51,234 | 1,54,673 | 1,51,234 | nil | nil | nil | nil | 88 |
| 33 | 37 | 36 | 33 | 0.9 | 1.1 | 0.9 | 0.8 | 1,58,095 | 1,66,579 | 1,55,685 | 1,76,069 | trace | nil | nil | nil | 185 |
| 26 | 24 | 28 | 24 | 0.6 | 0.8 | 0.8 | 1 | 1,55,678 | 1,56,789 | 1,55,769 | 1,55,655 | nil | nil | nil | nil | 134 |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 24 | 26 | 24 | 26 | 0.8 | 0.9 | 0.9 | 0.8 | 1,54,356 | 1,53,678 | 1,54,324 | 1,52,455 | nil | nil | nil | nil | 160 |
| 25 | 28 | 22 | 28 | 0.9 | 0.9 | 0.8 | 0.9 | 1,67,895 | 1,51,234 | 1,54,673 | 1,51,234 | trace | nil | nil | nil | 155 |
| 37 | 32 | 33 | 33 | 0.7 | 0.8 | 1.2 | 1 | 1,56,678 | 1,67,059 | 1,45,075 | 1,57,069 | nil | nil | nil | 1+ | 195 |
| 25 | 29 | 25 | 26 | 0.6 | 0.8 | 0.8 | 1 | 1,67,895 | 1,51,234 | 1,54,673 | 1,51,234 | nil | nil | nil | nil | 167 |
| 25 | 23 | 28 | 24 | 0.8 | 0.9 | 0.9 | 0.8 | 1,54,356 | 1,53,678 | 1,54,324 | 1,52,455 | trace | nil | nil | nil | 143 |
| 37 | 32 | 33 | 38 | 0.9 | 1 | 0.9 | 0.9 | 1,54,035 | 1,57,658 | 1,67,565 | 1,57,095 | nil | nil | nil | 2+ | 243 |
| 27 | 27 | 24 | 27 | 0.8 | 0.9 | 0.9 | 0.8 | 1,67,895 | 1,51,234 | 1,54,673 | 1,51,234 | nil | nil | nil | nil | 89 |
| 28 | 29 | 25 | 26 | 0.9 | 0.9 | 0.8 | 0.9 | 1,54,356 | 1,53,678 | 1,54,324 | 1,52,455 | trace | nil | nil | nil | 79 |
| 35 | 44 | 66 | 61 | 0.8 | 0.9 | 1 | 1.1 | 1,60,532 | 98,654 | 60000E | 1,00,654 | TRACE | 1+ | 4+ | 4+ | 727 |
| 26 | 24 | 28 | 24 | 0.6 | 0.8 | 0.8 | 1 | 1,55,678 | 1,56,789 | 1,55,769 | 1,55,655 | nil | nil | nil | nil | 89 |
| 24 | 26 | 24 | 26 | 0.8 | 0.9 | 0.9 | 0.8 | 1,54,356 | 1,53,678 | 1,54,324 | 1,52,455 | trace | nil | nil | nil | 167 |
| 25 | 28 | 22 | 28 | 0.6 | 0.8 | 0.8 | 1 | 1,67,895 | 1,51,234 | 1,54,673 | 1,51,234 | nil | nil | nil | nil | 155 |
| 24 | 33 | 32 | 28 | 0.9 | 0.8 | 1.1 | 0.9 | 1,76,676 | 1,71,059 | 1,56,775 | 1,67,769 | nil | trace | nil | 1+ | 192 |
| 23 | 28 | 24 | 25 | 0.9 | 0.9 | 0.8 | 0.9 | 1,55,678 | 1,56,789 | 1,55,769 | 1,55,655 | nil | nil | nil | nil | 132 |
| 28 | 29 | 25 | 26 | 0.9 | 0.9 | 0.8 | 0.9 | 1,54,356 | 1,53,678 | 1,54,324 | 1,52,455 | trace | nil | nil | nil | 79 |
| 29 | 27 | 27 | 24 | 0.6 | 0.8 | 0.8 | 1 | 1,67,895 | 1,51,234 | 1,54,673 | 1,51,234 | nil | nil | nil | nil | 134 |
| 32 | 36 | 36 | 40 | 0.8 | 0.9 | 1 | 1.2 | 1,56,735 | 1,67,558 | 1,56,265 | 1,65,095 | nil | nil | 1+ | 2+ | 256 |
| 25 | 23 | 28 | 24 | 0.8 | 0.9 | 0.9 | 0.8 | 1,67,895 | 1,51,234 | 1,54,673 | 1,51,234 | nil | nil | nil | nil | 123 |
| 26 | 24 | 28 | 24 | 0.6 | 0.8 | 0.8 | 1 | 1,55,678 | 1,56,789 | 1,55,769 | 1,55,655 | nil | nil | nil | nil | 133 |
| 33 | 53 | 65 | 66 | 7 | 0.8 | 1 | 1.1 | 1,60,456 | 1,60,423 | 99,657 | 67,567 E | TRACE | 2+ | 3+ | 4+ | 648 |
| 26 | 24 | 28 | 24 | 0.9 | 0.9 | 0.8 | 0.9 | 1,54,356 | 1,53,678 | 1,54,324 | 1,52,455 | nil | nil | nil | nil | 145 |
| 24 | 26 | 24 | 26 | 0.9 | 0.9 | 0.8 | 0.9 | 1,67,895 | 1,51,234 | 1,54,673 | 1,51,234 | trace | nil | nil | nil | 98 |
| 28 | 24 | 25 | 24 | 0.8 | 0.9 | 0.9 | 0.8 | 1,54,356 | 1,53,678 | 1,54,324 | 1,52,455 | nil | nil | nil | nil | 132 |
| 22 | 28 | 25 | 29 | 0.8 | 0.9 | 0.9 | 0.8 | 1,67,895 | 1,51,234 | 1,54,673 | 1,51,234 | nil | nil | nil | nil | 167 |
| 34 | 48 | 66 | 68 | 0.8 | 0.9 | 0.9 | 0.9 | 1,50,423 | 1,50,438 | 56000E | 86,007 | NIL | 2+ | 4+ | 4+ | 1409 |
| 28 | 24 | 25 | 26 | 0.9 | 0.9 | 0.8 | 0.9 | 1,55,678 | 1,56,789 | 1,55,769 | 1,55,655 | nil | nil | nil | nil | 143 |
| 29 | 25 | 26 | 24 | 0.8 | 0.9 | 0.9 | 0.8 | 1,54,356 | 1,53,678 | 1,54,324 | 1,52,455 | trace | nil | nil | nil | 135 |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 22 | 28 | 25 | 29 | 0.6 | 0.8 | 0.8 | 1 | 1,67,895 | 1,51,234 | 1,54,673 | 1,51,234 | nil | nil | nil | nil | 146 |
| 32 | 36 | 38 | 40 | 0.8 | 0.8 | 1.1 | 1.2 | 1,50,535 | 1,00,538 | 1,00,267 | 1,00,495 | nil | nil | 2+ | 3+ | 295 |
| 27 | 23 | 24 | 28 | 0.9 | 0.9 | 0.8 | 0.9 | 1,54,356 | 1,53,678 | 1,54,324 | 1,52,455 | nil | nil | nil | nil | 167 |
| 28 | 24 | 25 | 29 | 0.9 | 0.9 | 0.8 | 0.9 | 1,67,895 | 1,51,234 | 1,54,673 | 1,51,234 | trace | nil | nil | nil | 145 |
| 28 | 29 | 25 | 26 | 0.8 | 0.9 | 0.9 | 0.8 | 1,54,356 | 1,53,678 | 1,54,324 | 1,52,455 | trace | nil | nil | nil | 123 |
| 25 | 24 | 26 | 24 | 0.8 | 0.9 | 0.9 | 0.8 | 1,67,895 | 1,51,234 | 1,54,673 | 1,51,234 | nil | nil | nil | nil | 165 |
| 32 | 32 | 39 | 42 | 0.8 | 0.9 | 1.1 | 1.1 | 1,70,545 | 1,72,538 | 1,70,467 | 1,70,495 | nil | nil | 1+ | 2+ | 289 |
| 28 | 24 | 25 | 26 | 0.8 | 0.9 | 0.9 | 0.8 | 1,54,356 | 1,53,678 | 1,54,324 | 1,52,455 | nil | nil | nil | nil | 145 |
| 29 | 25 | 26 | 24 | 0.8 | 0.9 | 0.9 | 0.8 | 1,67,895 | 1,51,234 | 1,54,673 | 1,51,234 | trace | nil | nil | nil | 134 |
| 25 | 29 | 25 | 26 | 0.6 | 0.8 | 0.8 | 1 | 1,67,895 | 1,51,234 | 1,54,673 | 1,51,234 | nil | nil | nil | nil | 123 |
| 32 | 25 | 28 | 22 | 28 | 0.9 | 1 | 1.2 | 1,50,535 | 1,60,739 | 1,70,267 | 1,55,495 | nil | nil | 2+ | 3+ | 284 |
| 25 | 23 | 28 | 24 | 0.8 | 0.9 | 0.9 | 0.8 | 1,54,356 | 1,53,678 | 1,54,324 | 1,52,455 | nil | nil | nil | nil | 156 |
| 24 | 30 | 27 | 23 | 0.8 | 0.9 | 0.9 | 0.8 | 1,67,895 | 1,51,234 | 1,54,673 | 1,51,234 | trace | nil | nil | nil | 164 |
| 25 | 23 | 28 | 24 | 0.8 | 0.9 | 0.9 | 0.8 | 1,54,356 | 1,53,678 | 1,54,324 | 1,52,455 | trace | nil | nil | nil | 143 |
| 35 | 53 | 67 | 67 | 0.9 | 0.8 | 0.9 | 1 | 1,50,325 | 1,50,002 | 98,897 | 67,800E | NIL | 2+ | 2+ | 4+ | 654 |
| 24 | 28 | 24 | 25 | 0.6 | 0.8 | 0.8 | 1 | 1,67,895 | 1,51,234 | 1,54,673 | 1,51,234 | nil | nil | nil | nil | 147 |
| 32 | 36 | 38 | 40 | 0.8 | 0.8 | 1.1 | 1.2 | 1,55,535 | 1,50,538 | 1,50,267 | 1,50,495 | nil | nil | 2+ | 3+ | 138 |
| 25 | 24 | 26 | 24 | 0.6 | 0.8 | 0.8 | 1 | 1,55,678 | 1,56,789 | 1,55,769 | 1,55,655 | nil | nil | nil | nil | 89 |
| 24 | 26 | 24 | 24 | 0.8 | 0.9 | 0.9 | 0.8 | 1,54,356 | 1,53,678 | 1,54,324 | 1,52,455 | trace | nil | nil | nil | 139 |
| 25 | 23 | 28 | 24 | 0.8 | 0.9 | 0.9 | 0.8 | 1,67,895 | 1,51,234 | 1,54,673 | 1,51,234 | nil | nil | nil | nil | 87 |
| 24 | 28 | 24 | 25 | 0.9 | 0.9 | 0.8 | 0.9 | 1,67,895 | 1,51,234 | 1,54,673 | 1,51,234 | trace | nil | nil | nil | 153 |
| 32 | 32 | 39 | 42 | 0.8 | 0.9 | 1.1 | 1.1 | 1,60,545 | 1,62,538 | 1,60,467 | 1,65,495 | nil | nil | 2+ | 3+ | 333 |
| 26 | 24 | 28 | 24 | 0.6 | 0.8 | 0.8 | 1 | 1,55,678 | 1,56,789 | 1,55,769 | 1,55,655 | nil | nil | nil | nil | 133 |
| 24 | 26 | 24 | 26 | 0.8 | 0.9 | 0.9 | 0.8 | 1,54,356 | 1,53,678 | 1,54,324 | 1,52,455 | nil | nil | nil | nil | 147 |
| 25 | 28 | 22 | 28 | 0.6 | 0.8 | 0.8 | 1 | 1,67,895 | 1,51,234 | 1,54,673 | 1,51,234 | nil | nil | nil | nil | 154 |
| 32 | 36 | 38 | 40 | 0.8 | 0.8 | 1.1 | 1.2 | 1,50,535 | 1,50,538 | 1,50,267 | 1,50,495 | nil | nil | 2+ | 3+ | 290 |
| 24 | 25 | 24 | 26 | 0.6 | 0.8 | 0.8 | 1 | 1,54,356 | 1,53,678 | 1,54,324 | 1,52,455 | nil | nil | nil | nil | 134 |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 26 | 24 | 26 | 24 | 0.8 | 0.9 | 0.9 | 0.8 | 1,67,895 | 1,51,234 | 1,54,673 | 1,51,234 | nil | nil | nil | nil | 132 |
| 29 | 25 | 26 | 24 | 0.8 | 0.9 | 0.9 | 0.8 | 1,54,356 | 1,53,678 | 1,54,324 | 1,52,455 | trace | nil | nil | nil | 135 |
| 26 | 24 | 28 | 24 | 0.6 | 0.8 | 0.8 | 1 | 1,55,678 | 1,56,789 | 1,55,769 | 1,55,655 | nil | nil | nil | nil | 133 |
| 26 | 24 | 28 | 24 | 0.6 | 0.8 | 0.8 | 1 | 1,55,678 | 1,56,789 | 1,55,769 | 1,55,655 | nil | nil | nil | nil | 145 |
| 24 | 26 | 24 | 26 | 0.8 | 0.9 | 0.9 | 0.8 | 1,54,356 | 1,53,678 | 1,54,324 | 1,52,455 | nil | trace | nil | nil | 159 |
| 25 | 28 | 22 | 28 | 0.9 | 0.9 | 0.8 | 0.9 | 1,67,895 | 1,51,234 | 1,54,673 | 1,51,234 | nil | nil | nil | nil | 148 |
| 24 | 26 | 24 | 26 | 0.8 | 0.9 | 0.9 | 0.8 | 1,54,356 | 1,53,678 | 1,54,324 | 1,52,455 | nil | nil | nil | nil | 160 |
| 30 | 27 | 23 | 24 | 0.8 | 0.9 | 0.9 | 0.8 | 1,55,678 | 1,56,789 | 1,55,769 | 1,55,655 | nil | nil | nil | nil | 134 |
| 23 | 28 | 24 | 25 | 0.9 | 0.9 | 0.8 | 0.9 | 1,54,356 | 1,53,678 | 1,54,324 | 1,52,455 | nil | nil | nil | nil | 146 |
| 28 | 29 | 25 | 26 | 0.6 | 0.8 | 0.8 | 1 | 1,67,895 | 1,51,234 | 1,54,673 | 1,51,234 | nil | nil | nil | nil | 138 |
| 23 | 28 | 24 | 25 | 0.9 | 0.9 | 0.8 | 0.9 | 1,55,678 | 1,56,789 | 1,55,769 | 1,55,655 | nil | nil | nil | nil | 132 |
| 25 | 24 | 26 | 24 | 0.8 | 0.9 | 0.9 | 0.8 | 1,55,678 | 1,56,789 | 1,55,769 | 1,55,655 | nil | nil | nil | nil | 145 |
| 24 | 25 | 24 | 26 | 0.9 | 0.9 | 0.8 | 0.9 | 1,54,356 | 1,53,678 | 1,54,324 | 1,52,455 | nil | nil | trace | nil | 137 |
| 26 | 24 | 26 | 24 | 0.6 | 0.8 | 0.8 | 1 | 1,67,895 | 1,51,234 | 1,54,673 | 1,51,234 | nil | nil | nil | nil | 153 |
| 24 | 30 | 27 | 23 | 0.8 | 0.9 | 0.9 | 0.8 | 1,67,895 | 1,51,234 | 1,54,673 | 1,51,234 | trace | nil | nil | nil | 164 |
| 28 | 24 | 25 | 24 | 0.8 | 0.9 | 0.9 | 0.8 | 1,54,356 | 1,53,678 | 1,54,324 | 1,52,455 | nil | nil | nil | nil | 132 |
| 24 | 26 | 24 | 25 | 0.9 | 0.9 | 0.8 | 0.9 | 1,67,895 | 1,51,234 | 1,54,673 | 1,51,234 | nil | nil | nil | nil | 146 |
| 32 | 36 | 38 | 40 | 0.8 | 0.8 | 1.1 | 1.2 | 1,50,535 | 1,50,538 | 1,50,267 | 1,50,495 | nil | nil | 2+ | 3+ | 290 |
| 25 | 24 | 26 | 24 | 0.8 | 0.9 | 0.9 | 0.8 | 1,67,895 | 1,51,234 | 1,54,673 | 1,51,234 | nil | nil | nil | nil | 171 |

## PROFORMA

NAME OF THE PATIENT : OP NO:

AGE :

OCCUPATION :

ADDRESS :

COMPLAINTS :

PAST HISTORY :

PERSONAL HISTORY :

FAMILY HISTORY :

DRUG HISTORY :

**GENERAL EXAMINATION**:

Ht: Wt: BMI: BP: PR:

**SYSTEMIC EXAMINATION**:

CVS: RS:

ABD: CNS:

**INVESTIGATIONS :**

1) SERUM ALT
2) SERUM AST
3) SERUM CREATININE:
4) PLATELET COUNT
5) URINE PROTEIN
6) SOLUBLE FMS LIKE TYROSINE KINASE 1

# CONSENT FORM

Dr. K.Manjukarthikeyani post graduate student in the department of Biochemistry, Thanjavur medical college, Thanjavur is doing a Study of soluble fms like tyrosine kinase 1 as predictive marker of pre ecalmpsia in primigravida. The procedures has been explained to me clearly. I understand that there are no risks involved in the above procedures. I hereby give my consent to participate in this study. The data obtained here may be used for research and publication.

Signature :

Name:

Place: